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ANIMAL GENETICS AND BREEDING

ANIMAL FEEDS AND NUTRITION

ANIMAL PRODUCTION

REPRODUCTIVE PERFORMANCE OF LANDRACE AND YORKSHIRE SOWS FROM DIFFERENT GENETIC RESOURCES RAISED AT KBANG FARM, GIA LAI PROVINCE

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ABSTRACT

This study was conducted at Kbang farm, Kbang district, Gia Lai province from August 2021 to January 2024 from 802 sows: 415 Landrace (L) and 387 Yorkshire (Y) including 79 originated from Canada (CA), 51 from the USA (US), and 672 between Canada and USA (CA×US). The effect of Breed (L, Y), Origin (CA, US, CA×US), Season (Rainy, Dry), and Parity (l-4+) on reproductive traits, including age at first mating (AFM), age at first farrowing (AFF), duration of cycle (DC), number born (NB), number born alive (NBA), number of weaning (NW), birth weight (BW), litter weight at birth (LBW), weaning weight (WW) and litter weight at weaning (LWW). All records were from the purebred animals. The season, parity and origin affected all study traits ($P<0.05$) except WW between origins ($P=0.1962$); DC, NBA and LBW between seasons ($P>0.05$). NW, BW, LBW and LWW of Landrace were higher than those of Yorkshire. NBA, NW, WW and LWW in dry season were higher than those in rainy season ($P<0.05$). The increase in the NBA and NW in dry season led to decreased BW ($P<0.05$). Reproduction of CA×US sows was higher than those of CA and US.

Keywords: *Exotic breed, swine, reproduction, origin.*

1. INTRODUCTION

In recent years, different exotic pig breeds have been imported from different countries by various livestock companies in Vietnam. The reproductive performance of Landrace (L) and Yorkshire (Y) from different origins were reported by Trịnh Hồng Sơn *et al.* (2019), Nguyen Thi Hong Nhung *et al.* (2020) and Hà Xuân Bộ and Đỗ Đức Lực (2020). The effect of the combination of origins on the reproductive performance of L and Y in Northern Vietnam was published (Trịnh Hồng Sơn *et al.*, 2019). Reproduction of L and Y from different genetic resources was described in study of Đoàn Phương Thuý *et al.* (2015).

Breeding programs for purebreds play an important role in the genetic improvement of the next generation in livestock production. Importing high genetic resources from other countries is an important way to improve the performance of the livestock population. Clayton and Genesus are American and Canadian companies, in genetic improvement for the high-quality pig breeds in the world (Clayton, 2024, Genesus, 2024). According to the Mavin group, in 2021, 94 gilts (43 L and 51 Y), and 582 semen doses (272 L and 310 Y) were imported from Genesus, Canada to Kbang farm by the Mavin group. Similarly, the Mavin also imported additional genetics, including 54 gilts (29 L and 25 Y), and 867 semen doses (390 L and 477 Y) in 2022 from Clayton, USA to this farm. The genetics from Canada and the USA were used for purebred reasons with different combinations between origins. The initial results show that the pigs at Kbang farm develop well under industrial farming conditions. The objective of this research was to investigate the reproductive traits of L and Y sows and the factors affecting these traits.

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2. MATERIALS AND METHODS

2.1. Experimental design

The data was collected at Kbang farm, Kbang district, Gia Lai province from August 2021 to January 2024 from 802 sows (415 L and 387 Y) including 79 originated from Canada (CA), 51 from the USA (US), and 672 from the mating between CA and US (CA×US). The reproductive performance was age at first mating (AFM, day), age at first farrowing (AFF, day), duration of the cycle (DC, day), number born (NB, piglet), number born alive (NBA, piglet), number of weaning (NW, piglet), birth weight (BW, kg), litter weight at birth (LBW, kg), weaning weight (WW, kg), litter weight at weaning (LWW, kg). The number of piglets was counted while litter weight was recorded at birth and weaning at the age of 23.67±5.41 days. All records were from the purebred and were from the fourth parity and were marked as 4+. The season was divided by rainy (May to October) and dry (November to April of the next year).

2.2. Data analysis

Data were analyzed using SAS 9.1 software (2002) with the following statistical model. $Y_{ijklm} = \mu + B_i + O_j + B_i \times O_j + S_k + P_l + \varepsilon_{ijklm}$; *where: Y_{ijklm} = reproductive traits, μ = overall mean, B_i = effect of breed i (i=2: Landrace, Yorkshire), O_j = effect of origin j (j=3: CA, US, CA×US), S_k = effect of season k (k=2: Rainy, Dry), and P_l = effect of parity l (k=4: 1, 2, 3, 4+); ε_{ijklm} = random error*. The statistical parameters in the results section are number of observations (n), least square mean (LSM), and standard error (SE). Pairwise comparisons between LSMs were performed using Tukey's test.

3. RESULTS AND DISCUSSION

3.1. Effect of the fixed factors on reproductive performance

The season, parity and origin affected all study traits (P<0.05) except WW between origins (P=0.1962); DC, NBA and LBW between seasons (P>0.05). NW, BW, LBW and LWW were significantly different between L and Y (P<0.05). The coefficient of determination (R^2) ranged from 5.45 for NW to 44.87 for AFM (Table 1).

Table 1. Effect of Breed (B), Origin (O), Season and Parity

Trait	Breed	Origin	B*O	Season	Parity	R^2 (%)
AFM	0.5150	<.0001	0.1061	<.0001	-	44.87
AFF	0.0804	<.0001	0.4600	<.0001	-	24.48
DC	0.3225	<.0001	0.6635	0.1503	<.0001	14.71
NB	0.2910	<.0001	0.4346	0.0021	<.0001	11.54
NBA	0.1304	<.0001	0.1902	0.1397	<.0001	8.89
NW	0.0061	<.0001	0.0388	<.0001	0.0006	5.45
BW	0.0160	<.0001	0.1743	0.0269	<.0001	11.77
LBW	0.0261	<.0001	0.6310	0.7615	<.0001	9.90
WW	0.6463	0.1962	0.9038	<.0001	0.0007	6.86
LWW	0.0054	<0001	0.0585	<.0001	<.0001	10.42

3.2. Reproductive performance of Landrace and Yorkshire sows

Table 2. Reproduction performance of breeds

Trait	Landrace			Yorkshire		
	n	LSM	SE	n	LSM	SE
AFM (day)	415	247.41	1.56	387	248.86	1.59
AFF (day)	415	375.83	2.95	387	383.22	3.02
DC (day)	360	155.89	2.09	317	158.84	2.36
NB (piglet)	777	12.51	0.19	708	12.80	0.21
NBA (piglet)	766	11.20	0.18	695	10.81	0.20
NW (piglet)	701	9.84[a]	0.11	643	9.40[b]	0.13
BW (kg)	737	1.79[a]	0.02	669	1.73[b]	0.02
LBW (kg)	737	19.60[a]	0.34	669	18.53[b]	0.38
WW (kg)	701	6.22	0.05	642	6.19	0.06
LWW (kg)	701	61.11[a]	0.80	642	57.97[b]	0.89

In the same row, LSMs with different letter are significantly different (P<0.05)

The NB and NBA was similar between breeds (P>0.05) while at weaning, NW of L (9.84) was higher (P=0.0051) than that of Y sows (9.40). BW, LWB, and LWW of Landrace were also higher than those of Yorkshire (Table 2). However, WW did not differ between the two breeds (P=0.6463). BW and WW found similarities between L and Y (Nguyễn Ngọc Thanh Yên *et al.*, 2018). BW of L was heavier than that of Y (Hà Xuân Bộ and Đỗ Đức Lực, 2020; Nguyen Thi Hong Nhung *et al.*, 2020). Inversely, NW, LBW, and LWW of L were lower than Y in the study by Nguyen Thi Hong Nhung *et al.* (2020). These traits were not different between the two breeds (Trịnh Hồng Sơn *et al.*, 2019; Hà Xuân Bộ and Đỗ Đức Lực, 2020). Our results are

consistent with conclusions from Đoàn Phương Thuý *et al.* (2015).

3.3. Reproductive performance of Landrace and Yorkshire sows according to origin

The reproductive traits according to origin are presented in Table 3. NB, NBA, NW and LWW were not different between CA and CA×US (P>0.05). These values of CA and CA×US were higher than those of US (P<0.05). Inversely, BW of US was higher than that of CA and CA×US (P<0.0001). BW was lowest in CA (1.61), followed by CA×US (1.73), and highest in the US (1.95). The WW was not significantly different between origin (P=0.6463). The high reproduction of CA×US in comparison to CA and the US might relate to sows born in Vietnam and from parents with different origins. The mating of different lines might be also the result of performance improvement due to the recombination between dominant alleles. The effect of pig origin on reproduction in Vietnam was mentioned (Luc *et al.*, 2013). These authors confirm that the gilts imported had a lower reproduction than those born from the imported animals. The combinations of mating between origins (France and US) affected reproductive traits (Trịnh Hồng Sơn *et al.*, 2019).

Table 3. Reproduction performance Landrace and Yorkshire by origin

Trait	CA			CA×US			US		
	n	LSM	SE	n	LSM	SE	n	LSM	SE
AFM (day)	79	267.39^{a}	2.16	672	222.85^{c}	0.70	51	251.97^{a}	2.67
AFF (day)	79	399.80^{a}	4.09	672	349.25^{b}	1.33	51	390.63^{a}	4.99
DC (day)	205	174.55^{a}	2.40	370	140.57^{c}	2.42	102	156.67^{b}	3.39
NB (piglet)	290	14.05^{a}	0.23	1042	13.80^{ab}	0.19	153	10.09^{b}	0.32
NBA (piglet)	289	11.80^{a}	0.21	1023	12.25^{a}	0.17	149	8.95^{b}	0.30
NW (piglet)	270	9.89^{a}	0.14	931	10.02^{a}	0.11	143	8.88^{b}	0.19
BW (kg)	280	1.61^{c}	0.02	983	1.73^{b}	0.02	143	1.95^{a}	0.03
LWB (kg)	280	19.07^{b}	0.40	983	21.15^{a}	0.33	143	16.97^{c}	0.57
WW (kg)	269	6.11	0.06	931	6.25	0.05	143	6.23	0.08
LWW (kg)	269	60.11^{a}	0.96	931	62.68^{a}	0.78	143	55.34^{b}	1.31

3.4. Reproductive performance of breed sows by parity

The DC gradually decreased (P<0.0001) from the second (174.54) to the fourth parity (137.84). NB increased (P<0.0001) regularly from the first (11.25) to the fourth parity (13.56). Contrarywise, NBA was lowest in the first and increased from second and was highest in the fourth parity (Table 4). BW and LBW were lowest in the first, then increased and stable from second to fourth parity. For NW, WW and LWW, the trend was observed to increase from the first to the second and then decrease from the third party (P<0.01). Our results are consistent with findings from Nguyễn Ngọc Thanh Yên *et al.* (2018). Parity significantly affected the reproductive traits of L and Y except BW and WW (Nguyen Thi Hong Nhung *et al.*, 2020).

Table 4. Reproductive performance Landrace and Yorkshire by parity

Trait	Parity 1			Parity 2			Parity 3			Parity 4+		
	n	LSM	SE	n	LSM	SE	n	LSM	SE	n	LSM	SE
DC (day)	-	-	-	388	174.54^{a}	2.07	189	159.71^{b}	2.53	100	137.84^{c}	3.76
NB (piglet)	802	11.25^{b}	0.19	390	12.42^{b}	0.22	191	13.26^{ab}	0.28	102	13.69^{a}	0.41
NBA (piglet)	791	9.79^{b}	0.18	384	10.88^{ab}	0.20	185	11.52^{ab}	0.27	101	11.83^{a}	0.38
NW (piglet)	714	9.46^{b}	0.11	365	10.01^{a}	0.13	176	9.38^{b}	0.17	89	9.62^{ab}	0.25
BW (kg)	771	1.65^{b}	0.02	360	1.81^{a}	0.02	180	1.77^{a}	0.03	95	1.82^{a}	0.04
LBW (kg)	771	15.64^{b}	0.34	360	19.66^{a}	0.39	180	19.58^{a}	0.51	95	21.38^{a}	0.74
WW (kg)	713	6.10^{b}	0.05	365	6.36^{a}	0.06	176	6.23^{ab}	0.08	89	6.12^{ab}	0.11
LWW (kg)	713	57.31^{b}	0.79	365	63.29^{a}	0.91	176	58.67^{b}	1.19	89	58.89^{ab}	1.76

3.5. Reproductive performance of Landrace and Yorkshire sows by season

The NBA, NW, WW and LWW in dry season were higher than those in rainy season (P<0.05). The increase in the NBA and NW in dry season led to decreased BW (P<0.05). LBW was similar between the two seasons (P=0.7615). Effect of season on reproduction of L and Y was reported by Nguyễn Ngọc Thanh Yên *et al.* (2018); Nguyen Thi Hong Nhung *et al.* (2020). The season did not affect the NB and BW and WW (Nguyen Thi Hong Nhung *et al.*, 2020). Nguyễn Ngọc Thanh Yên *et al.* (2020) confirmed that reproduction of L and Y was not different between season (P>0.05), except BW of Y (P= 0,0498) and LWW of L (P=0,0374).

Table 5. Reproductive performance L and Y by season

Trait	Rainy		Dry			
	n	LSM	n	LSM	n	LSM
AFM (day)	407	243.61^{b}	407	243.61^{b}	407	243.61^{b}
AFF (day)	407	373.03^{b}	407	373.03^{b}	407	373.03^{b}
DC (day)	403	159.30	403	159.30	403	159.30
NB (piglet)	813	12.34^{b}	813	12.34^{b}	813	12.34^{b}
NBA (piglet)	795	10.86	795	10.86	795	10.86
NW (piglet)	713	9.34^{b}	713	9.34^{b}	713	9.34^{b}
BW (kg)	759	1.78^{a}	759	1.78^{a}	759	1.78^{a}
LBW (kg)	759	19.12	759	19.12	759	19.12
WW (kg)	713	5.95^{b}	713	5.95^{b}	713	5.95^{b}
LWW (kg)	713	55.43^{b}	713	55.43^{b}	713	55.43^{b}

4. CONCLUSIONS

The season, parity and origin affected all study traits (P<0.05) except WW between origins (P=0.1962); DC, NBA and LBW between seasons (P>0.05).

NW, BW, LBW and LWW of Landrace were higher than those of Yorkshire. Reproduction of CA×US sows was higher than those of CA and US.

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THE EVOLVING LANDSCAPE OF ANIMAL NUTRITION AND FEED TECHNOLOGY IN KOREA

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ABSTRACT

South Korea's livestock industry is crucial for national food security. We highlight the growing demand for high-quality animal feed and the trend towards precision feeding and functional feed additives. Sustainability concerns are driving research into Eco-friendly feed production and reducing the environmental footprint. Advancements in feed technology include novel processing techniques and the utilization of alternative feed ingredients like insect protein. We discuss the challenges of rising feed costs, limited land, and consumer demands for antibiotic-free meat. Finally, we explore the exciting opportunities presented by new feed ingredients, bio-engineering for improved feed efficiency, and the potential of artificial intelligence for personalized animal nutrition plans. However, over 90% of Korea's feed ingredients are imported, making the industry vulnerable to global market fluctuations and supply chain disruptions and increasing domestic production of feed ingredients to reduce reliance on imports. Develop strategies to effectively utilize locally available resources, such as food byproducts and agricultural waste, in animal feed formulations. Besides, ongoing efforts are required to enhance the quality and value of feed through innovative processing techniques and functional feed additives. By addressing these limitations and pursuing continuous improvement, Korea can further strengthen its animal nutrition and feed technology sector, ensuring a sustainable, efficient, and environmentally responsible livestock industry.

Keywords: *Livestock, animal feed, feed formulations, environment.*

1. INTRODUCTION

Animal nutrition plays a vital role in the overall health and productivity of livestock. In Korea, the growing demand for high-quality animal products has spurred the need for advancements in feed technology and nutritional strategies. South Korea's animal feed industry is an integral part of its agricultural sector, crucial for supporting livestock production including poultry, swine, and cattle. Over recent years, the industry has seen significant shifts driven by technological advancements, increased environmental awareness, and changing consumer preferences toward sustainable and ethical farming practices.

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Animal feed plays a vital role in the agricultural and livestock sectors, underpinning the health and productivity of animals bred for various uses. This feed consists of a specialized blend of ingredients designed to fulfill the nutritional needs of animals, incorporating essential nutrients, vitamins, and minerals to promote their growth, health, and overall performance. Typically, animal feed includes a mixture of grains like corn, wheat, and barley, along with protein sources such as soybean meal, fishmeal, or meat and bone meal. These components are meticulously combined to cater to the specific dietary needs of different types of animals, whether they are being raised for meat, milk, eggs, or other products. The significance of high-quality animal feed is critical, as it directly influences the animals' health and the quality of the products derived from them for human consumption. Proper nutrition through balanced feed is essential for optimizing growth rates,

boosting immune systems, and mitigating disease risks. Additionally, animal feed is integral to sustainable agriculture by enabling efficient use of resources and reducing the environmental impact of livestock farming.

The most commonly consumed meats in the world are poultry and pork. Global pork consumption has increased by 77% from 63.5 million tons in 1990 to 113 million tons in 2022, whereas poultry and beef saw a 287 and 49% increase during the same period, respectively (Kim *et al*., 2024). In 2023, global pork production exceeded 120 million metric tons. China led the world in pork production, contributing over 55 million metric tons to the total. South Koreans' consumption of meat has continuously increased over the years. In 2023, pork consumption reached 1.4 million tons, marking a 1.92% increase compared to the previous year.

Traditionally, Korean livestock farming relied heavily on conventional feedstuffs such as grains and forage. However, with the industrialization of agriculture in the mid-20th century, there was a shift towards more scientifically formulated feeds. The introduction of balanced rations and nutrient-enriched feed marked the beginning of modern animal nutrition in Korea. This review will emphasize the growing demand for high-quality animal feed and the trend toward precision feeding and functional feed additives.

2. CURRENT TRENDS IN THE ANIMAL FEED INDUSTRY IN KOREA

The South Korean feed industry is pivotal in sustaining the country's robust livestock sector, which is crucial for fulfilling domestic needs for meat, dairy, and eggs. As of 2024, the industry faces various important trends and challenges that define its current landscape. The growing demand for high-quality meat and dairy products in South Korea is propelling the livestock sector, consequently boosting the feed ingredients market. This growth is driven by shifts in dietary preferences and increasing incomes, which have heightened the demand for animal feed to sustain a growing livestock population. Moreover, as South Koreans consume more meat, particularly pork, and poultry, the need for feedstock, including corn and soybean meal utilized in animal feed production, has also risen.

2.1. Compound feed production

Korea's animal feed production has gradually grown over the past decade, surpassing 20 million tons for the first time in 2019, driven by the increase in pig sows, breeding stocks, and the potential for calf production. The feed ingredients market in the country is primarily propelled by the demand for livestock feed, which is specifically formulated for cattle, pigs, and sheep. These feed ingredients are necessary to optimize growth rates, improve nutritional value, and enhance overall health in livestock. The increasing demand for high-quality meat and dairy products in South Korea is driving growth in the livestock sector, which in turn is stimulating the feed ingredients market. Cattle feed makes up a substantial part of the feed market, serving the needs of dairy and beef cattle nationwide. Poultry feed constitutes a significant segment, fueled by the rising consumption of chicken and eggs. The growth in commercial poultry farming has sparked an increase in demand for specialized feed formulations designed to accelerate growth and enhance egg production. Additionally, swine feed is essential in the livestock sector, ensuring efficient growth and health maintenance. In 2023, compound feed production in South Korea reached approximately 21.5 million tons, with 33% allocated for swine, 28% for poultry, and 27% for beef cattle (Ministry of Agriculture, Food and Rural Affairs - MAFRA, 2023). The compound feed production for swine totaled about 7 million tons, followed by poultry feed at 6.1 million tons. Additionally, the total compound feed

for cattle reached nearly 7 million tons, combining the amounts used for both dairy and beef cattle (Table 1).

Table 1. Compound feed production in 2023

Livestock species	Compound feed production (tons)
Poultry	6,102,270.31
Pig	7,080,691.44
Dairy	1,248,342.95
Beef	5,717,046.16
Other	1,345,125.18

2.2. Compound feed production by province

The production of compound feed in Korea is not uniformly distributed across the country. It is concentrated in specific provinces, reflecting regional differences in livestock farming, industrial infrastructure, and access to raw materials. Compound feed production in Korea continued to be a cornerstone of the agricultural sector, with significant contributions from key provinces such as Gyeonggi, Chungcheong, Jeolla, and Gyeongsang (KFA, 2023). In Korea, there are 79 feed mills with a combined daily production capacity of 21,496 tons (Table 2). The provinces with the most feed mills are Jeolla, Chungcheong, and Gyeonggi, hosting 19, 18, and 16 mills respectively.

Table 2. Location of feed mills in Korea

Location	Number of feed mill	Production capacity/day
Daegu Metropolitan City	1	330
Daejeon Metropolitan City	1	480
Incheon Metropolitan City	9	3,100
Busan Metropolitan City	1	330
Gangwon-do	1	300
Gyeonggi-do	16	3,247
Gyeongsangnam-do	6	1,600
Gyeongsangbuk-do	5	2,210
Jeollanam-do	2	270
Jeollabuk-do	17	5,019
Chungcheongnam-do	11	2,701
Chungcheongbuk-do	7	1,634
Jeju-si	2	195
Total	79	21,416

2.3. Compound feed raw materials usage

Animal feeds mostly use traditional crops such as corn, soy, or wheat to provide necessary nutrients at an economical cost (EFSA, 2008). Over 80% of imported raw materials were used in compound feed. Korea's total consumption of compound feed raw materials reached 21.46 million tons, with grains (e.g. corn and wheat) being the leading ingredients used in compound feed production, accounting for 11.54 million tons (Table 3). Corn remains the most significant component in compound feed, accounting for over 50% of the total feed composition in Korea, with most of the supply relying on imports from the United States, Brazil, and Ukraine (USDA-FAS, 2024). Corn reflects 9.26 million tons and feed wheat amounted to 1.86 million tons of feed consumption. Corn is valued for its high energy content, making it a staple in feeds for poultry, swine, and cattle (Maner *et al.,* 1985). Wheat is used as a secondary energy source and is particularly popular in regions where corn is less available or more expensive. South Korea imports wheat from countries such as Australia, the United States, and Canada. The use of wheat in compound feed has seen a slight increase, driven by the need for diversification of energy sources amid fluctuating corn prices.

Table 3. Consumption of feed ingredients in 2023

Feed ingredient	Quantity (million tons)
Grains	11.541
Yellow corn	9.259
Feed wheat	1.857
Others	0.425
Brans	2.054
Animal protein	0.212
Vegetable protein	5.437
Inorganic matters	0.931
Others	1.288
Total	21.461

Another component of compound feed is animal-based single feeds, which are derived from various sources such as fish meal, meat and bone meal, blood meal, and other by-products from the meat processing industry. Animal-based single feeds are essential components in the diet of livestock, providing vital nutrients such as protein, fats, and essential amino acids that contribute to

the overall health and productivity of the animals. South Korea's production of animal-based single feed decreased by 10.70% in 2022, followed by a further decline of 4.23% in 2023 compared to the previous year. Notably, fish meal production has fallen by 25% over the past five years. The Korean government, along with industry stakeholders, has implemented strict regulations to ensure that these feeds are free from contaminants and are produced under hygienic conditions. Stricter regulations on livestock farming environments and disease prevention measures have disrupted the supply of animal by-products. Research is ongoing in Korea to explore alternative protein sources, such as plant-based and insect-based feeds, which could complement or partially replace traditional animal-based single feeds. This is driven by the need to diversify feed sources and reduce reliance on conventional animal-based ingredients. In recent years, South Korea has seen a noticeable shift towards sustainability in agriculture and animal husbandry, paralleling global trends. This shift includes an increasing reliance on plant-based single feeds, which are derived from a single type of plant or plant product and used in the diets of livestock. These feeds are crucial for providing a balanced intake of nutrients while also addressing environmental concerns associated with traditional animal-based feeds. Plant-based single feeds are typically derived from crops such as soybeans, corn, barley, wheat, and other oilseeds and grains. These feeds are rich in carbohydrates, proteins, fiber, and essential fatty acids, making them vital components of livestock diets. The production of plant-based feed in Korea dropped by 10% over the years. However, as efforts to utilize plant-based by-products, such as mushroom compost and garlic waste, as feed resources gain momentum, a government pilot project is set to launch this year, focusing on large-scale feed resource utilization of plant-based byproducts from the Garak Agricultural Products Market. This development is expected to significantly enhance the utilization of discarded waste as feed in the future.

The compound feed industry in South Korea faces several challenges in raw material procurement. South Korea imports a substantial amount of its animal feed ingredients, such as corn and soybean meal. The cost and availability of these ingredients are heavily influenced by international trade agreements and global commodity prices. In line with this, the increasing consumer awareness of animal welfare and sustainable farming practices has shifted preferences towards products from animals raised on quality feed. This shift has motivated livestock producers to procure and utilize high-quality feeds. The heavy reliance on imports makes the industry vulnerable to global market fluctuations, including price volatility and supply chain disruptions. Additionally, there is a growing demand for non-GMO and organic feed options, which adds another layer of complexity to raw material sourcing. Sustainability is also becoming a key concern, with increasing pressure on the industry to adopt practices that reduce environmental impact. This includes sourcing from more sustainable agricultural practices and reducing the carbon footprint associated with importing raw materials.

3. TECHNOLOGICAL ADVANCEMENTS IN FEED FORMULATION

3.1. Advances in nutrient profiling and formulation

Recent developments in nutrient profiling and formulation have revolutionized the way feed is designed. Modern feed formulation technologies utilize sophisticated software and algorithms to precisely balance nutrient levels, ensuring that feed meets the specific needs of different livestock species. Advanced software tools

analyze vast amounts of data, including ingredient composition, nutrient requirements, and cost considerations, to create optimal feed formulations. One notable advancement is the use of precision feeding technologies. This approach involves tailoring feed formulations to meet the specific needs of different animal species, breeds, and even individual animals. Advanced technologies such as near-infrared spectroscopy (NIR) and computerized feed formulation software are being increasingly adopted to optimize nutrient intake and improve feed efficiency. These systems employ sensors and data analytics to monitor individual animal performance and adjust feed rations in real time. This approach not only improves feed efficiency but also minimizes waste and reduces feed costs (Moss et al., 2021; Gajaweera *et al.,* 2023). The demand for feed additives and supplements that boost animal immunity and overall health is fueling innovation in feed formulations. Additionally, the South Korean animal feed industry has adopted technological advancements in feed formulation, production, and delivery. Modern feed mills in South Korea utilize automation and precision technologies to efficiently produce high-quality, nutritionally balanced feeds.

3.2. Improved ingredient processing techniques

Advancements in ingredient processing have also contributed to more effective feed formulations. Technologies such as extrusion, pelleting, and micronization enhance the digestibility and nutrient availability of feed ingredients. Extrusion, for example, uses high pressure and temperature to modify the physical and chemical properties of feed ingredients, making them more digestible and nutritious (Lancheros *et al.,* 2022). Micronization, which involves grinding ingredients to a very fine powder, improves nutrient absorption and enhances feed efficiency. This technique is particularly useful for incorporating high-fiber ingredients and by-products into feed formulations without compromising nutrient density (Chuang *et al.,* 2021).

3.3. Integration of functional additives

The incorporation of functional additives into feed formulations represents a significant technological advancement. These additives, including probiotics, prebiotics, and enzymes, are designed to improve animal health, enhance growth performance, and optimize nutrient utilization. Probiotics, for example, support the development of beneficial gut microflora, while prebiotics stimulate the growth of these beneficial microbes (Shoukry *et al*., 2023). Enzymes are another critical addition to modern feed formulations. Feed enzymes are widely utilized in livestock diets to improve nutrient digestion and boost their growth performance. They break down complex feed components, such as fiber and phytic acid, increasing the availability of nutrients and improving overall feed efficiency. This not only benefits animal health but also contributes to environmental sustainability by reducing the amount of undigested feed waste (Sureshkumar *et al*., 2023).

3.4. Sustainable feed formulation practices

Sustainability is a growing focus in feed formulation, driven by the need to reduce the environmental impact of livestock production. Advances in technology have facilitated the development of more sustainable feed ingredients and formulations. For instance, research into alternative protein sources, such as insect meal and algae, offers promising solutions to reduce reliance on traditional feed ingredients and minimize environmental footprints (Pomar *et al.,* 2021). Additionally, technological advancements in feed processing and formulation enable more efficient use of resources, reducing waste and improving overall sustainability. Technologies that optimize ingredient

utilization and minimize feed waste contribute to a more sustainable and environmentally friendly feed production system (Akintan *et al.,* 2024). Moreover, stricter environmental concerns and regulations surrounding livestock farming have led to the adoption of sustainable feed production practices and innovations designed to minimize the environmental impact of the livestock sector.

4. DEVELOPMENT OF FUNCTIONAL FEEDS

Functional feed ingredients enhance productivity and vitality by improving digestibility, maintaining and stabilizing beneficial gut microflora, and positively affecting the environment (Ndudzo *et al*., 2023). The use of functional feeds, which are intended to improve animal health and performance beyond basic nutrition, is gaining popularity. These feeds often include probiotics, prebiotics, enzymes, and other additives that enhance gut health, boost immunity, and improve overall productivity. Korea has seen a rise in the development of functional feeds, which include additives like probiotics, enzymes, and herbal extracts. These components are designed to boost the immune system of animals, enhance growth rates, and improve feed efficiency. Research and development in this area are heavily supported by both governmental and private sectors, aiming to reduce the use of antibiotics in livestock through natural alternatives. Probiotics, plant phytogenic compounds, and prebiotics have the potential to replace antimicrobials as environmental-friendly therapeutics and growth promoters.

4.1. Types and effects of functional feed materials

The Korean livestock industry has seen substantial growth and transformation, largely driven by advancements in feed technology, including the integration of functional feed materials. These materials are designed to enhance animal health, improve growth performance, and increase the overall efficiency of livestock production.

Probiotics

Probiotic feed is increasingly customized to meet the specific needs of different livestock species and stages of development. Probiotics in animal feed help balance gut microbiota, strengthen immunity, and increase resistance to stress (Wang *et al*., 2021; Ma *et al*., 2023). Common probiotic strains used in livestock feeds include *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*. The market offers a wide range of probiotic products with various strains and functional properties. In Korea, probiotic feed manufacturers are actively expanding into international markets. Advances in genomics and precision livestock farming will enable tailored probiotic solutions based on individual animal characteristics.

Prebiotics

Prebiotics are non-digestible food components that promote the growth of beneficial microorganisms in the intestines (Yasmin *et al*., 2015). These substances, which typically include fructooligosaccharides (FOS), mannanoligosaccharides (MOS), and galactooligosaccharides (GOS), help improve digestion and nutrient absorption and strengthen the immune system. Probiotics promote the growth of beneficial intestinal microorganisms, enhancing the effects of probiotics (Ma *et al*., 2023).

Synbiotics

Synbiotics contain both probiotics and prebiotics and are used in animal nutrition. This blend synergistically enhances the host's health by supporting the survival and colonization of live microbial dietary supplements in the gastrointestinal (GI) tract. It selectively fosters the growth and/or activates the metabolism of specific beneficial bacteria, thereby boosting the overall welfare of the host (Markowiak and Śliżewska, 2018). A combination of *Bifidobacterium* or *Lactobacillus* genus bacteria

with FOS in synbiotic products seems to be the most popular. Current data on the effects of synbiotics on animal health are limited and necessitate further research. Nevertheless, the available evidence clearly demonstrates the effective synergistic interaction between probiotics and prebiotics in reducing populations of gastrointestinal bacterial pathogens.

Herb extracts

Herb extracts, which are substances derived from plants, are added to animal feed to enhance overall performance and product quality. These plant extracts comprise various compounds, with the active secondary plant metabolites primarily being isoprene derivatives and flavonoids. Herb extracts have been proven to offer significant benefits within the digestive system, including laxative effects, prevention of flatulence, stimulation of digestive secretions, and enhanced enzyme activity (Alem, 2024). Curcumin has been used in animal feed due to its strong antioxidant activity to remove oxygen radicals (Goel *et al*., 2008; Sahin *et al*., 2012). Additionally, it helps in the reduction of lipid peroxidation by stabilizing cell organelles through the reduction of oxygen radicals (Griesser *et al*., 2011; Khan *et al*., 2012) and have protective effect against lipid peroxidation process with anti-inflammatory, antioxidant and antibacterial effects (Negi *et al*., 1999; Khalil *et al*., 2012).

Amino acids

Amino acids are building blocks for proteins for animals, which were traditionally classified as nutritionally essential or nonessential, based on growth or nitrogen balance (Wu, 2014). It was previously believed that all "nutritionally nonessential amino acids (NEAA)" were adequately synthesized in the body to support maximum growth and optimal health. NEAAs, such as glutamine, glutamate, proline, glycine, and arginine, are crucial for regulating gene expression, cell signaling, antioxidative responses, fertility, neurotransmission, and immunity. Additionally, glutamate, glutamine, and aspartate serve as key metabolic fuels for the small intestine, crucial for maintaining its digestive function and protecting the integrity of the intestinal mucosa. Therefore, animal diets need to include all NEAAs to enhance survival, growth, development, reproduction, and health. Moreover, the inclusion of NEAAs should be considered when revising the "ideal protein" concept currently used in formulating diets for swine and poultry.

Amino acids in animal feed have become a crucial element of livestock nutrition. The demand for amino acids in animal feed has steadily risen over the past few years, and this trend is expected to continue throughout the years. Most animals lack the ability to synthesize amino acids on their own, which has led to the growing popularity of amino acids in animal feed over the past few years. Additionally, animal nutritionists worldwide have increasingly turned to the use of amino acids in animal feed to meet the rising demand for livestock products and to enhance feed efficiency at a lower cost. In recent years, significant advancements have allowed animal nutritionists to better assess the internal activities of the rumen. As a result, stakeholders in the animal feed amino acids market are anticipated to combine amino acids with protein to reduce diet costs and enhance livestock production (Reference). Low protein feed formulations with amino acids are used to enhance intestinal health, and reduce costs, glucose metabolism, nitrogen utilization, and growth performance (Duarte *et al*., 2024). Lysine, an essential amino acid, holds more than 50% share of the global animal amino acid market in 2018. Notably, the demand for lysine-based amino acids in animal feed has risen, particularly in swine and poultry applications, due to growing awareness of the various nutritional

benefits these products provide. In addition, the use of lysine has become popular among livestock farmers for its multiple benefits for animal health as well as to save costs in replacing expensive raw materials such as soybean meal.

Minerals

Mineral-based single feed has decreased by approximately 30% over the past five years. This decline is partly attributed to the increased reliance on imported calcium phosphates, such as dicalcium phosphate, from China. Most mineral-based single feeds have seen a decrease, with the exception of trace minerals. While the domestic production of these feeds, typically used as supplementary ingredients, is expected to continue its decline, the market for high-value products like organic trace minerals is anticipated to grow. Notably, organic trace minerals have been incorporated into feed since the 1970s, starting with the adoption of Metal Proteinate as an AAFCO standard. This was followed by the introduction of Metal amino acid Chelate standards in 1988 and Metal amino acid Complex standards in 1990. Zinc is another mineral used for animal feed. It is an important element for normal growth, bone development, feather formation, and immunity in chickens (Salim *et al.,* 2011). When added to the feed in the form of nanoparticle zinc oxide, it increases the absorption rate of zinc in organs, bones, and muscles, thereby improving meat quality and reducing stress (Song *et al.,* 2009). Moreover, when added in large amounts, zinc intoxication occurs and is excreted through manure (Swain *et al.,* 2016).

Vitamins

Vitamin C is essential for certain animals, including humans, subhuman primates, and guinea pigs, who cannot synthesize it themselves and must obtain it from their diet. However, many other mammals, such as ruminants, swine, horses, dogs, and cats, are capable of producing ascorbic acid (AsA) from glucose in their liver (Matsui, 2012). Vitamin C is involved in metabolism through electron donation to oxidized molecules (Khan, 2011) and leukocyte production process (Ružic *et al.,* 2020). Several studies showed that vitamin C can improve immunity and antioxidant function in stressful situations (Brake, 1989; Padayatty *et al.,* 2003). Moreover, vitamin C can enhance nutrient digestibility through the protection of intestinal tissue (Attla *et al.,* 2009).

5. LOW PROTEIN AND LOW METHANE FEED FOR LIVESTOCK

In 2018, South Korea's total greenhouse gas emissions reached 727.6 million metric tons (MMT) on a carbon dioxide equivalent (CO_2e) basis, with the agricultural sector contributing 3.4% or 24.7 MMT of these emissions. The country has implemented robust strategies to mitigate methane emissions, recognizing methane as a potent greenhouse gas with significant environmental impacts. The MAFRA recently announced key initiatives to drive greenhouse gas (including methane) emission reduction in the agricultural sector. In 2022, the MAFRA announced the 'Livestock Industry Environment Improvement Plan'. This announcement provided the public and industry stakeholders with detailed information about new initiatives and tools designed to facilitate the transition towards a more sustainable livestock industry. This policy not only aligns with South Korea's carbon neutrality goals but also with global environmental standards (USDA-FAS, 2022).

The MAFRA opted to transition to low protein and low methane feed as key strategies for reducing greenhouse gas emissions in the livestock industry. Low protein feed contains reduced amounts of high-protein ingredients such as soybean meal and leads to less production of animal

waste and greenhouse gases, including ammonia and nitrogen. This initiative to transition to low-protein formulation in feeds is expected to reduce the consumption of protein ingredients like soybean meal in swine and poultry feed, with projected reductions reaching up to 70,000 metric tons. Meanwhile, low methane feed refers to a feed that contains additives (methane-reducing agents) that restrict the growth of bacteria that are responsible for generating methane gas through enteric fermentation. In this regard, the MAFRA newly established some regulations in October 2023, which stated that the standard for "low-methane feed" must have a significant methane reduction effect of 10% or more compared to conventional feed (control group) (MAFRA, 2023). Most methane-reducing agents reduce methane production by inhibiting the growth of methanogens that produce methane in the rumen or by blocking the methane production process caused by methanogens. In addition, the use of substances that suppress hydrogen, a precursor of methane production, by promoting the production of volatile fatty acids, which are used as the main energy source for ruminant livestock, is also being suggested as a major method. Currently, the most effective substances for reducing methane emissions in livestock include red seaweed and sea squirt-both also used as food-and the chemical agent 3-Nitrooxypropanol (3-NOP), all of which have demonstrated significant methane mitigation capabilities (Tseten *et al.,* 2022; Choi *et al.,* 2022). Additionally, various other substances such as probiotics, plant extracts, organic acids, tannins, nitrates, and neutral fats have also been identified for their potential to reduce methane emissions. Additionally, the Korean government has introduced the "Low-Carbon Livestock Certification System," which awards certification to farms that achieve a reduction in greenhouse gas emissions of 10% or more compared to average farm emissions by incorporating low-carbon livestock technologies into their production processes (KREI, 2024).

6. CHALLENGES AND FUTURE OUTLOOK

Despite the advancements, the Korean animal nutrition and feed technology sector faces several challenges, such as the high costs of advanced feed formulations and the need for continuous research to keep up with changing animal nutritional requirements Additionally, the need for continuous research and development to keep up with global trends and regulatory requirements poses a significant burden on the industry. Moreover, demographic changes and shifts in dietary habits, such as the decline in rice consumption, are influencing the demand for certain feedstocks. However, these challenges also present opportunities. The increasing focus on sustainable and alternative feed sources could open new markets and reduce dependency on traditional feed ingredients. Moreover, the adoption of precision nutrition and smart feeding technologies could lead to significant improvements in feed efficiency and animal productivity, offering a competitive advantage to Korean livestock producers.

Overall, the South Korean feed industry in 2024 is marked by a focus on sustainability, technological innovation, and adapting to changing consumer and regulatory demands. Emerging technologies such as artificial intelligence (AI) and machine learning offer promising prospects for further enhancing feed efficiency and reducing environmental impacts. Furthermore, the development of novel feed ingredients, such as cultured proteins and insect-based feeds, is expected to gain momentum. These innovations could not only address the challenges of feed scarcity and environmental impact but also meet the evolving consumer demands for ethical and sustainable animal products.

7. CONCLUSION

The evolving landscape of animal nutrition and feed technology in Korea reflects a dynamic industry that is adapting to global trends and challenges. Through innovation and the adoption of new technologies, Korea is well-positioned to enhance its livestock production systems and meet the growing demand for high-quality, sustainable animal products. As the industry continues to evolve, it will be crucial for stakeholders to embrace these changes and capitalize on the opportunities they present.

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EFFECTS OF YEAST FERMENTATION ON THE QUALITY OF SOYBEAN MEAL AND PEANUT MEAL

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ABSTRACT

The aim of this study was to determine the good time point and substrates of soybean meal (SBM) and peanut meal (PNM) fermented with *Saccharomyces cerevisiae.* Thus, parameters of pH, yeast cells population, chemical compositions of fermented products after incubation were measured and evaluated. As a result, after 72h of incubation, *(i)* yeast cells population of SBM quickly reached near the highest value at 24h while that of PNM had to wait until 72h, *(ii)* the protein content of PNM increased by 3.68% while that of PNM only increased by 1.75% (P<0.05). However, the crude protein content of fermented SBM was always quite high compared to that of PNM fermentation (51.47 vs 44.54%, respectively) (P<0.05). Besides, most other chemical components such as Ash and EE decreased, especially CF of SBM (-46.04%) (P<0.05); and *(iii)* a statistically significant interaction between substrates (SBM and PNM) and moistures (50% and 70%) was also noted, in which the CP content of PNM incubated at 50% moisture with the highest value. The findings ground a foundation for the application of incubation of SBM and PNM with *S. cerevisiae* in animal production practices and for subsequent expansion studies.

Keywords: *Fermentation process, Saccharomyces cerevisiae, chemical composition.*

1. INTRODUCTION

Saccharomyces cerevisiae - the common yeast species in bread and sourdough generally regarded as safe and presenting an excellent biotechnological strategy for bioconversion of cereals into nutrient-rich fermented food rations for human, is called the solid-state fermentation (Yafetto *et al.*, 2022). It has been used in different production industries to produce wine, beer, bread, chocolate, bioethanol, and biofuel as well as in recombinant proteins (Parapouli *et al.*, 2020; Yang *et al.*, 2024). It is also reported in detail that the solid-state fermentation increased the protein content of commercial soybean meals by 13.65%, increased the total of hydrolyzed amino acids by 16.27%, and decreased phytic acid and trypsin inhibitor (Hassaan *et al.*, 2015). The fermentation of PNM by *S. cerevisiae* inoculation may improve volatile fatty acid production and increase the proportion of even acids. It means that an acetic acid concentration of 10,797.09 mg/l, which was 1.82 times higher than that obtained with only the PNM fermentation, was found with the *S. cerevisiae* inoculum: PNM ratio of 0.15 g/g (Zhang *et al.*, 2020). Therefore, it is recommended that fermentation of plant protein sources with *S. cerevisiae* increases their value and efficiency while reducing the cost of feed mills (Hassaan *et al.*, 2015). This is even more scientifically significant for SBM because they contain anti-nutritional factors. Those are also the objectives of this study.

2. MATERIALS AND METHODS

2.1. Samples

Samples of the SBM and PNM were collected from a feed mill in Dong Nai province and used as substrates for the fermentation. *S. cerevisiae* obtained from a commercial baker's yeast (Instant Success Dry Yeast, Lesaffre, France).

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2.2. Fermentation process

Substrates of the SBM and PNM were mixed with distilled water to the required relative moisture contents of 50 and 70%. The yeast activity was stimulated by adding 1g of dry yeast powder to 100ml of activator solution (10% glucose) and incubated for 24h at room temperature (27-30^{o}C). To study the effects of the different substrates (SBM and PNM) and initial moisture contents (50 and 70%), the fermentation was performed by inoculating 2% (v/w) of the activated yeast solution with a cell density of about 10^{8}CFU/ml into the substrates.

The mixture was placed in a tight plastic bag for fermentation at room temperature. After 24, 48 and 72h, the fermented samples were dried at 55^{o}C till the moisture content was less than 10%. The dried samples were ground through a 3mm sieve and stored at cold temperature (2-4^{o}C).

2.3. Experimental parameters

The population of *S. cerevisiae* during fermentation was counted every 24h by plating on the MRS agar while pH was determined using the portable pH meter (Horiba PH210, Japan).

The dry matter (DM), ash, organic matter (OM), CP, ether extract (EE) and crude fiber (CF) contents of the substrates and fermented samples were analyzed in conformity with the guidelines of the Association of Official Analytical Chemists (AOAC, 2005).

2.4. Statistical analysis

Data were statistically analyzed with the general linear model (GLM) of Minitab 16 software. Means were compared using Tukey's test at a 5% significance level ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1. Yeast cells population and pH

During 72h of incubation, *(i)* the yeast cell population increased at both humidity levels (50% and 70%) and two substrate materials (SBM and PNM) (Figure 1 and 2). All increases were linear. However, there was a rapid increase within 24h after incubation for SBM and a gradual increase within 72h for PNM. These increases approached 10x10^{7}CFU/g at 72h. This means that the population increment in SBM was negligible after 24h of incubation. Therefore, incubation of SBM with *S. cerevisiae* should not be extended to shorten harvest time and increase investment efficiency in practical application; *(ii)* along the increase of yeast cell population, the pH value rapidly decreased due to the speed and time sufficient to produce an amount of acid corresponding to the experimental factors (Figures 3 and 4). The rapid decrease in pH was concentrated in the first 24h and between 48h and 72h after incubation while the pH during the 24-48h period did not have major fluctuations. The narrowest range of pH fluctuations during incubation was found in the treatment with PNM substrate and 70% moisture.

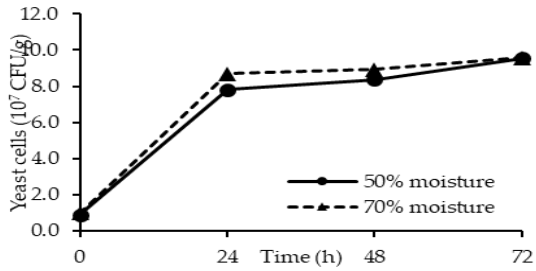

Figure 1. Growth curve of yeast in SBM during 72h fermentation

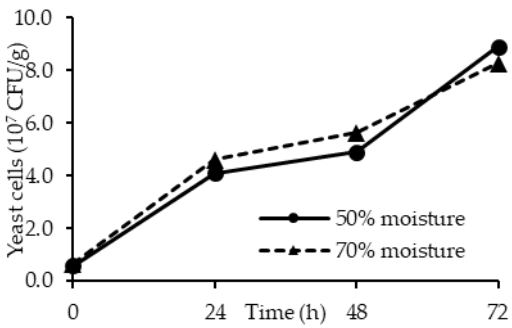

Figure 2. Changes of yeast population during 72h fermentation of PNM

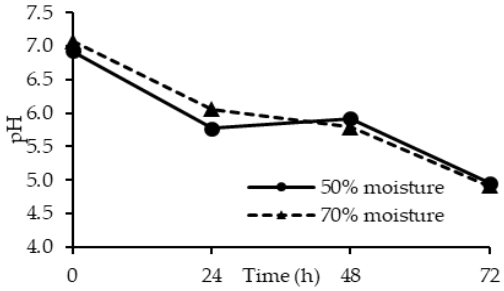

Figure 3. Changes of pH during 72h fermentation of SBM

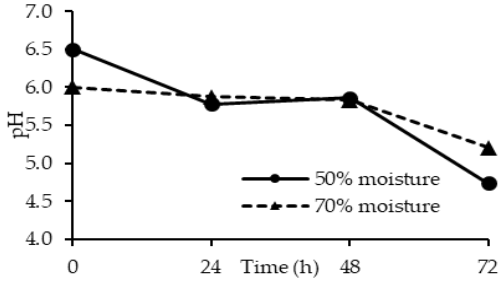

Figure 4. Changes of pH during 72h fermentation of PNM

3.2. Chemical compositions of fermented products

The results in table 1 indicated that the DM (89.98 vs 88.66), CP (50.58 vs 44.09) and EE (7.80 vs 6.31) contents of SBM were higher than those of PNM before fermentation (P<0.05). This trend was similar for both CP (51.47 vs 44.54) and EE (7.23 vs 6.14) at 72h after incubation (Table 2) (P<0.05). Additionally, changing two moisture levels of 50% and 70% during fermentation led to the decrease of the CP (49.10 vs 46.91) and EE (7.02 vs 6.35) contents, respectively. In particular, there was a statistically significant interaction between substrate and moisture for the CP content of PNM where the higher one was found at 50% (46.89 vs 44.52) of moisture.

Table 1. The composition of SBM and PNM

Composition (%)	**SBM**	**PNM**	**SEM**	**P**
DM	89.98	88.66	0.33	0.047
Ash	7.23	7.74	0.16	0.083
CP	50.58^{a}	44.09^{b}	0.32	0.000
EE	7.80^{a}	6.31^{b}	0.15	0.002
CF	4.82	4.11	0.30	0.171

Table 2. Chemical composition after 72h fermentation

Factors			**DM**	**Ash**	**CP**	**EE**	**CF**
Substrate	SBM		84.44	6.11	51.47^{a}	7.23^{a}	2.60
	PNM		84.97	6.59	44.54^{b}	6.14^{b}	3.10
Moisture	50%		85.32	6.41	49.10^{a}	7.02^{a}	2.99
	70%		84.10	6.29	46.91^{b}	6.35^{b}	2.71
Substrate × Moisture	SBM	50%	84.13	6.14	51.30^{a}	7.64	3.02^{a}
		70%	84.75	6.08	51.64^{a}	6.82	2.18^{ab}
	PNM	50%	86.50	6.68	46.89^{b}	6.41	2.96^{ab}
		70%	83.44	6.50	44.52^{c}	5.88	3.24^{b}
SEM	Substrate		0.96	0.19	0.27	0.20	0.18
	Moisture		0.96	0.19	0.27	0.20	0.18
	Subs×Moi		1.36	0.27	0.39	0.28	0.25
P	Substrate		0.702	0.095	0.000	0.001	0.060
	Moisture		0.379	0.673	0.000	0.026	0.279
	Subs×Moi		0.191	0.831	0.000	0.621	0.038

Note: Means within a column of each factor followed by different superscripts are significantly different at 5% (P<0.05).

Table 3. Changes SBM, PNM after 72h fermentation

Factors			**DM**	**Ash**	**CP**	**EE**	**CF**
Substrate	SBM		-6.15	-15.48	1.75^{b}	-7.31	-46.04^{b}
	PNM		-4.16	-14.80	3.68^{a}	-2.58	-24.64^{a}
Moisture	50%		-4.47	-14.37	3.89^{a}	-0.20^{a}	-32.70
	70%		-5.85	-15.91	1.53^{b}	-9.68^{b}	-37.98
Substrate × Moisture	SBM	50%	-6.50	-15.08	1.42^{b}	-2.07	-37.37^{ab}
		70%	-5.81	-15.88	2.08^{b}	-12.55	-54.71^{b}
	PNM	50%	-2.43	-13.66	6.37^{a}	1.66	-28.02^{a}
		70%	-5.89	-15.94	0.99^{b}	-6.82	-21.25^{a}
SEM	Substrate		1.07	2.53	0.39	2.82	3.86
	Moisture		1.07	2.53	0.39	2.82	3.86
	Subs×Moi		1.52	3.58	0.55	3.99	5.46
P	Substrate		0.205	0.851	0.002	0.250	0.001
	Moisture		0.373	0.671	0.000	0.028	0.345
	Subs×Moi		0.189	0.839	0.000	0.806	0.039

Interestingly, all CP concentrations increased regardless of substrate factors, moisture levels, or interactions between them while other components like DM, Ash, EE (except interaction between substrate and moisture of PNM noted 1.66) and CF were reduced (P<0.05). It is clear that *(i)* on different substrates, the CP content of SBM (1.75) was lower than that of PNM (3.68), *(ii)* while the CP content at 50% moisture (3.89) level was found to be higher than the remaining one (1.53) or showed the highest

value (6.37) in the interaction between the substrate PNM and 70% moisture level. SBM had the greatest decrease in DM (-6.15). This was due to the large loss of ash (-15.48), EE (-7.31) and mainly CF (-46.04). Actually, statistically significant differences in the CF content between substrates or in the interaction between substrate and moisture were found ($P<0.05$). The loss of the CF content of SBM was higher than that of PNM (-46.04 vs -24.64). Also, on the SBM substrate and at 70% humidity, the CF value deficiency was at its highest (54.71), which was in a strong agreement with Hassaan *et al.* (2015). It may be due to the fiber composition and structure of SBM being suitable for *S. cerevisiae* consumption or its easy degradation by fermentation while the increase in protein of the fermented products could include the contribution of the yeast protein.

Some previous studies indicated that *(i)* PNM contained protein, carbohydrates, minerals, vitamins, and small amounts of polyphenols and fiber and has long been used as a feed in animal production industries. After inoculation with *S. cerevisiae*, the anaerobic fermentation of PNM mainly produced even-chain volatile fatty acids (mainly acetic acid and n-butyric acid. In the early stage of fermentation, inoculation of *S. cerevisiae* enhanced protein dissolution efficiency and degradation rate, and completely degraded soluble glycogen (Zhang *et al.*, 2020); *(ii)* fermentation of SBM with *S. cerevisiae* for 48h could improve the nutritive value of SBM, especially protein and amino acids. It may be due to the high production of the cell mass of yeast and consequently the production of protein within the yeast population (Hassaan *et al.*, 2015); *(iii)* the reduction in the CF content of fermented SBM with *S. cerevisiae* may be due to the secretion of various enzymes, which degraded CF and complex polysaccharides (Belewu *et al.*, 2011). They further clarified the mechanism for the data bias in this study.

3. CONCLUSION

Fermentation of SBM and PNM with *S. cerevisiae* did not only help increase their biological value but could also convert anti-nutrients into compounds beneficial to animal health although research had not been proven yet. This promises a potential replacement of animal raw materials such as fishmeal - which is foul-smelling in the final product of the livestock value chain and is quite expensive - with fermented plant products in the future.

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PROTEIN ENRICHMENT OF CASSAVA BAGASSE WITH *SACCHAROMYCES CEREVISIAE* AND *SACCHAROMYCOPSIS FIBULIGERA* FOR USING AS CATTLE FEED

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ABSTRACT

The aim of this study was to evaluate the ability to enrich protein of cassava bagasse (CSB) by solid state fermentation with two yeast strains *Saccharomyces cerevisiae* SAC3 and *Saccharomycopsis fibuligera* BMQ908. The experiment was divided into three groups: ĐC (naturally fermented CSB or unenrichment), TN1 (protein-enriched CSB with two yeast strains) and TN2 (protein-enriched CSB with two yeast strains and enzyme, urea at 3 levels of of 21.7, 32.6 and 43.5 g/kg (equivalent to 1.0, 1.5, 2.0%N of DM). CSB was added with 5% of yeast culture solution (cell density reach ≥10^9CFU/ml). Moisture content of CB was adjusted to 60% w/v and pH 5-5,5, then fermented at 30^{o}C for 7 days. Samples were collected at 0, 1, 3, 5, 7 days of fermentation to determine crude protein (CP), true protein (TP), and total yeast number. The results showed that the CP and TP contents of CB were effectively improved after 5 days fermentation with *S. cerevisiae* SAC3 and *Sac. fibuligera* BMQ908 in TN1. The CP and TP contents were increased from 2.2 to 6.61%, and from 1.06 to 4.39% respectively, which corresponding to the highest number of total yeast at 5 days of fermentation (282.67x10^6 CFU/g). The CP and TP contents reached the highest level in enzyme treated group (27.66 and 9.67%, respectively). The TP content increased to 8,45 and 8,27% in urea treated groups CT1 and CT2 over the 5 days of fermentation.

Keywords: *Cassava bagasse, Saccharomyces cerevisiae, Saccharomycopsis fibuligera, protein enrichment.*

1. INTRODUCTION

Currently, the use of cassava bagasse (CSB) in cattle feeding remains limited due to its low crude protein (CP) content (1-4%) and relatively high cyanide levels (Iyayi and Losel, 2001). However, CSB can be utilized as an energy source due to its relatively high cellulose (20-30%) and starch (30-40%) contents (Yun *et al*., 2019). Therefore, if appropriate processing technologies are employed, particularly those that enhance the protein content of CSB, this material could become a promising feed ingredient. It could help reduce the reliance on imported protein sources such as soybean meal and fish meal, thus lowering feed costs. Fermentation technology using selected microbial strains has been demonstrated by research to be the most effective method for protein enrichment of cassava (Ubalua, 2007; Boonnop *et al*., 2009; Kaewwongsa *et al*., 2011; Khejornsart, 2021). Various microorganisms, including *Trichoderma pseudokoningii*, *Rhizopus oligosporus*, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae*, *Candida utilis*, etc., have the ability to convert the fiber and starch in cassava flour and bagasse into microbial protein, thereby increasing the protein content and improving the quality of these materials (Saelim *et al*., 2008; Bayitse *et al*., 2015; Khejornsart, 2021). Among these, the yeasts *Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera* are commonly applied in single-cell protein production and in the protein enrichment of agricultural by-products such as CSB, cassava peel, and corn (Kaewwongsa *et al*., 2011; Polyorach *et al*., 2013). The rumen microbiota of ruminants can efficiently utilize inorganic nitrogen sources like urea; therefore, supplementing CSB with inorganic nitrogen during fermentation with yeast can significantly

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improve its quality and provide a nitrogen source for the rumen microbiota. This paper presents the results of using a mixture of two yeast strains, *S. cerevisiae* and *Sac. fibuligera*, supported by fiber-degrading enzymes and varying levels of urea supplementation, to enrich the protein content of CSB for use in cattle feeding.

2. MATERIALS AND METHODS

2.1. Materials

*Yeast **Strains*:** One strain of *S. cerevisiae* SAC3 and one strain of *Sac. fibuligera* BMQ 908, selected and identified through gene sequencing methods at the Food Industry Institute.

***Cassava Bagasse*:** Fresh (FCSB) and dried cassava bagasse (DCSB) were collected from Duong Lieu, Hoai Duc, Hanoi.

***Commercial Enzyme*:** KEMZYMETM (Kemin) containing cellulase (12,396 U/g), xylanase (54,509 U/g), α-amylase (1,235 U/g), and β-glucanase (2,286 U/g).

***Hansen broth Medium*:** Composed of Peptone (10 g/l), Glucose (50 g/l), KH_2PO_4 (3 g/l), and $MgSO_4 \cdot 7H_2O$ (2-5 g/l), with a pH of 6.0±0.2. The medium was sterilized at 121°C for 15min.

2.2. Experimental setup

Cassava Bagasse Treatment: Fresh cassava bagasse was dried at 60°C for 48hrs, then ground into small particles (1-2mm). The experiment was designed as shown in table 1.

Table 1. Experimental design for protein enrichment of cassava bagasse

Experimental factors	DC	TN1	TN2
Enzyme Treatment	None	None	Addition of 0.1%
Fermentation Method	Natural fermentation	Fermentation with two selected yeast strains	Fermentation with two selected yeast strains
Fermentation Support Substances	None	None	60g molasses and urea at three levels (CT1, CT2, CT3)
Fermentation Duration	7 days	7 days	7 days

In the control group (DC), the CSB, after treatment, was spread on a tray to a thickness of 5cm, water was added to achieve a moisture content of approximately 60%, and it was left to ferment naturally at 30°C for 7 days.

Both the TN1 and TN2 groups were fermented using inoculum cultures of the two yeast strains *S. cerevisiae* and *Sac. fibuligera*. However, unlike TN2, the TN1 group did not use enzymes or fermentation support substances during the experiment.

Preparation method for the yeast inoculum: Each yeast strain was cultured separately in Hansen broth medium at 30°C for 24-48hrs. The two strains were mixed together in a 1:1 ratio prior to inoculation. The yeast inoculum must be free of contamination and have a cell density ≥10^9 CFU/ml for each strain.

Enzyme treatment method (TN2): The moisture content of the CSB was adjusted to 60%, and 0.1% (based on DM) of KEMZYMETM enzyme was added before fermentation for 24hrs. This was done to aid in the breakdown of fiber and starch during the fermentation of CSB. After enzyme treatment, the CSB was mixed with molasses and urea according to the following formulas:

CT1: 1kg CSB+21.7g urea (equivalent to 1.0%N based on DM)+60g molasses

CT2: 1kg CSB+32.6g urea (equivalent to 1.5% N based on dry matter)+60g molasses

CT3: 1 kg CSB+43.5g urea (equivalent to 2.0% N based on dry matter)+60g molasses

Fermentation method: After treatment, the CSB in the TN1 and TN2 groups was spread into trays with a diameter of 50cm, evenly distributed to a thickness of 5cm, and then 5% of the yeast inoculum was added. The mixture was thoroughly mixed to achieve a moisture content of approximately 60%, and the trays were covered with a thin, sterilized cloth to allow airflow. Fermentation was carried out at 30°C for 7 days. Samples were taken to analyze protein content at 0, 1, 3, 5

and 7 days of cultivation. The experiment was repeated three times.

The results will be evaluated based on:

Comparison of the protein enrichment ability of CSB with the mixture of the two yeast strains (TN1, TN2) and the control (natural fermentation).

Assessment of the impact of supporting factors on protein enrichment with yeast between TN1 and TN2 via the evaluation of the interaction of three experimental factors: enzyme treatment (A); addition of fermentation support substances (B); fermentation time (C).

2.3. Evaluation of fermentation indicators

Sensory evaluation of the quality of fermented CSB: Observation of color, odor, texture, and mold on the surface.

The pH of the fermented feed was determined according to the method of Hristov and Jones (2000) using a pH meter (Mettler Toledo).

Sampling method followed TCVN 13052:2021; The samples were analyzed at the Central Laboratory, Faculty of Animal Science, Vietnam National University of Agriculture.

DM was quantified according to TCVN 4326-2007; CP and TP were quantified using the Kjeldahl method (TCVN 4328-2001); CP was quantified according to TCVN 4329-2007; Lipid was quantified according to TCVN 4331-2007; Minerals were quantified according to TCVN 4331-2007; Starch content was quantified using a Micro Phazir AG (Thermo Scientific).

Yeast cells was determined by the dilution method and colony counting on agar plates.

2.4. Data analysis

The data were processed using R software version 4.2.2 (Team, 2022). Descriptive statistical parameters of the study indicators include: Sample size (n), least squares means (LSM), Standard deviation (SD), and standard error of the mean (SEM). Comparisons between pairs of means were performed using Tukey's Honest Significant Difference test. Two-way ANOVA was used to analyze the effects of urea or enzyme supplementation levels and sampling days on the CP and TP indicators based on the statistical model: $Yijk = \mu + UEi + Nj + UEi*Nj + \varepsilon ijk$. *Where, Yijk: Represents the CP and TP indicators; μ: Population mean; UEi: Effect of the ith level of urea supplementation (i=3; 1, 1.5 and 2) or the ith enzyme level (i=with and without); Nj: Effect of the j^{th} day (j=4: 1, 3, 5, 7); UEi*Nj: Interaction between urea or enzyme and sampling day; ϵijk: Random error.*

3. RESULTS AND DISCUSSION

3.1. Chemical composition of cassava bagasse before fermentation

The results in table 2 indicate that cassava pulp is a low-nutrient material. The CP content of CSB is low, ranging from 1.98% (FCSB) to 2.04% (DCSB). The lipid content is also low, only between 0.19 and 0.45%. However, the residual starch content in CSB is relatively high, ranging from 32.71% (FCSB) to 33.42% (DCSB). The fiber content of CSB varies between 17.68% (FCSB) and 18.19% (DCSB).

Table 2. Chemical composition of CSB

Analytical parameter	FCSB	DCSB
DM (%)*	15.72	87.59
Crude Protein (% DM)	1.98	2.04
Total Minerals (% DM)	1.31	1.47
Lipid (% DM)	0.19	0.45
Crude Fiber (% DM)	17.68	18.19
Starch (% DM)	32.71	33.42

According to Nguyen Van Phu *et al.* (2015), the protein content of CSB is relatively low, at 1.87% DM. The authors recommend that when using CSB as feed for ruminants, it is essential to supplement with a protein-rich feed to balance the energy and protein content in the ration.

3.2. Protein enrichment in cassava bagasse with yeast

Using *S. cerevisiae* and *Sac. fibuligera* with a 5% supplementation level for solid-

state fermentation of CSB has improved the quality of the bagasse. The results in table 3 indicate that after 7 days of fermentation, the naturally fermented CSB without yeast supplementation (control) showed a negligible increase in CP content (2.19-2.98%). In contrast, the CSB fermented with a mixture of the two yeast strains exhibited a significant improvement in protein content. The CP value reached 6.61%, and the TP content reached 4.39% after 5 days of fermentation. The yeast cells in the experimental group TN1 were highest at day 5 of fermentation (282.67 million cells/g), which corresponds to the peak levels of both CP and TP content. Wild yeast strains were also found in the control CSB but in very low quantities, with a slower growth rate, resulting in only minor improvements in protein content.

Table 3. Protein enrichment of cassava bagasse with S. cerevisiae and Sac. fibuligera (n=3)

Fermentation duration (days)	**CP** (%DM)		**TP** (%DM)		**∑Yeast cell** (10^6 CFU/g)	
	DC	***TN1***	***DC***	***TN1***	***DC***	***TN1***
0	$2,19^a$	$2,20^a$	$1,05^a$	$1,06^a$	0,00026	34,67
1	$2,23^b$	$3,25^a$	$1,08^b$	$2,14^a$	0,00030	63,33
3	$2,42^b$	$4,55^a$	$1,21^b$	$2,71^a$	0,00035	103,00
5	$2,77^b$	$6,61^a$	$1,38^b$	$4,39^a$	0,00078	282,67
7	2.98^b	$5,54^a$	$1,46^b$	$2,45^a$	0,00128	238,00

In the same row, mean values with different letters are statistically significantly different (P<0.05)

Sac. fibuligera is a yeast with the ability to efficiently hydrolyze starch present in CSB into sugars due to its α-amylase and glucoamylase enzymes. Along with *S. cerevisiae*, it uses these sugars to proliferate and accumulate biomass. Sira *et al.* (2007) reported that cassava, after peeling, drying, and grinding, contains up to 86.25% starch, with amylose constituting up to 28.75%. Amylose is water-soluble and easily hydrolyzed by the amylase and glucoamylase enzymes of yeast. The starch utilization efficiency of yeast ranges from 50-55%, and the conversion ratio of starch to cellular protein is approximately 1:3. The authors indicate that during the fermentation of cassava flour with *Candida tropicalis* yeast, 40% of the starch is utilized by the yeast, resulting in a 12% increase in CP content compared to the initial amount.

The improved protein content of cassava flour and CSB after fermentation with yeast in this study can be attributed to the rapid increase in yeast cell count or single-cell protein biomass during the fermentation process (Oboh, 2002). Additionally, the increase in protein content is also related to the yeast's ability to produce various extracellular enzymes, such as amylase, linamarase, and cellulase, into the cassava flour and CSB during fermentation by *S. cerevisiae* (Oboh and Akindahunsi, 2003).

3.3. Effect of fermentation support factors on protein enrichment of cassava bagasse with yeast

The results in table 4 indicate that with the support of fiber-degrading enzymes prior to fermentation, the CP and TP content of CSB increased significantly compared to untreated samples (20.58 vs. 4.43% and 7.24 vs. 2.55%), reaching their highest levels after 5 days of fermentation (times). The fiber in CSB is primarily neutral detergent fiber (NDF), which is resistant to degradation by yeast. Therefore, pre-treatment with enzymes enhances the yeast's ability to break down fiber, improving protein enrichment efficiency as cellulase and xylanase enzymes convert cellulose and xylan into glucose. Yeast utilizes the glucose to grow and accumulate biomass, thereby increasing protein content. While the high fiber content in CSB does not affect the digestibility for ruminants, the use of fiber-degrading enzymes improves digestibility and absorption in monogastric livestock.

The results evaluating the impact of urea supplementation on protein content show

that the CP content was highest in formulations CT2 and CT3 (21.64 and 23.56%). However, the TP content was highest in CT1 and CT2, at 8.45 and 8.27% on a dry matter basis (DMB). Ali *et al.* (2009) reported that urea supplementation enhances the ability of yeast to accumulate protein-rich biomass. However, the addition of excessive urea can have the opposite effect, inhibiting yeast growth (Polyorach et al., 2013). In CT3, with a high urea supplementation level (43.5 g/kg CSB), although the CP content was the highest, the TP value was very low and tended to decrease over fermentation time. This result can be explained by the high urea concentration creating a hypertonic environment, causing yeast cells to lose water, shrink, and become smaller, thereby inhibiting yeast growth. The yeast cell number did not increase during fermentation. From a sensory perspective, the fermenters corresponding to these formulations fermented slowly, and the fermented material still had a urea odor during fermentation. This led to a decrease in TP content and a reduction in protein quality.

Table 4. Effect of fiber breakdown enzyme, urea, times

Item	Evaluation	CP (%)	TP (%)
Enzyme treatment (A)	No treatment (TN1)	4,43^{b}	2,55^{b}
	With treatment (TN2)	20,58^{a}	7,24^{a}
	SEM	0,465	0,215
	P	<0.001	<0,001
Urea supplement levels (B)	CT1 (21,7g urea/kg CSB)	17,16^{c}	8,45^{a}
	CT2 (32,6g urea/kg CSB)	21,64^{b}	8,27^{a}
	CT3 (43,5g urea/kg CSB)	23,56^{a}	4,99^{b}
	SEM	0,11	0,07
	P	<0,001	<0,001
Times, days (C)	0	16,39^{e}	4,68^{e}
	1	17,13^{d}	5,22^{d}
	3	20,41^{c}	7,40^{c}
	5	26,51^{a}	10,52^{a}
	7	23,51^{b}	8.37^{b}
	SEM	0,15	0,08
	P	<0,001	<0,001
Interaction levels	AxB	0.001	0.001
	AxC	0.001	0.001
	BxC	0.001	0.001
	AxBxC	0.001	0.001

In this study, as the non-protein nitrogen (NPN) content increases, the TP content decreases, and vice versa. Chumpawadee and Soychuta (2009) reported that the NPN content of CSB fermented with yeast decreased significantly compared to naturally fermented CSB. When using cassava flour as feed for dairy cattle, a lower NPN content in the feed can help reduce the NPN content in the milk. Therefore, a urea supplementation level of 21.7-32.6 g/kg CSB is optimal, as this range achieves the highest TP content.

The CP and TP content of CSB increased progressively over fermentation time, reaching their maximum at 5 days of fermentation (26.51 and 10.52%, respectively). This is due to the fermentation process, where yeast utilizes inorganic nitrogen sources and molasses to increase cell count and accumulate protein-rich biomass (Oboh, 2002). These findings are consistent with previous reports. Oboh (2006) reported that the protein content of cassava peel fermented by solid-state fermentation with a mixture of *Saccharomyces cerevisiae* and *Lactobacillus spp.* increased from 8.2% to 21.1%. Boonnop *et al.* (2009) used baker's yeast to ferment cassava slices and fresh cassava bagasse with a urea supplementation of 48 g/kg. After 5 days of fermentation at 25°C, the crude protein content of cassava slices increased from 3.4% to 32.5% DMB (a 9.6-fold increase), and the fresh cassava bagasse increased from 3.2 to 21.1% DMB (a 6.6-fold increase). Similarly, Kaewwongsa et al. (2011) reported the highest CP and TP contents of 31.6 and 29.0% DMB, respectively, when fermenting cassava flour with 5% *Sac. cerevisiae* for 5 days.

Thus, the results of protein enrichment in CSB are significantly influenced by factors enzyme treatment before fermentation, urea supplementation levels, and fermentation time. All three factors, as well as their interactions, affect the CP and TP content of cassava bagasse.

3.4. Organoleptic evaluation of fermented cassava bagasse in TN2

After 1 day of fermentation, the CSB retained its original gray-brown color across

all formulations. After 3 days of fermentation, the color of the bagasse became darker. Upon touching, the bagasse felt warm and soft, porous, non-slimy, and emitted a pleasant, slightly sour smell in groups CT1 and CT2. At 5 days of fermentation, the bagasse in CT1 and CT2 had a dark gray-brown color, acid, slight alcoholic smell, and felt soft, warm and not slimy. The pH value of the CSB tended to decrease throughout the fermentation process. After 5 days of fermentation, the pH value dropped to below 5 in CT1 and CT2 (4.72 and 4.88) and continued to decrease to 4.61-4.65 after 7 days, resulting in a strong sour and alcoholic smell and a dark brown color. In contrast, due to the high level of urea supplementation in CT3, the bagasse still had a noticeable urea odor. After 7 days of fermentation, the bagasse in CT3 tended to clump together and felt wet and sticky. No mold was observed in any of the fermented CSB formulations.

Table 5. Sensory evaluation of protein-enriched fermented cassava bagasse according to times

Parameters		1 day	3 days	5 days	7 days
CT1	Color	Gray-brown	Dark gray-brown	Dark gray-brown	Dark brown
	Texture	Soft, porous	Soft, porous	Soft, porous	Soft, a litte wet
	Odor	Raw material odor	Slight acid, aromatic	acid, slight alcoholic	Strong sour, alcoholic
	Mold	-	-	-	-
	pH	5.56	5.37	4.72	4.61
CT2	Color	Gray-brown	Dark gray-brown	Dark gray-brown	Dark brown
	Texture	Soft, porous	Soft, porous	Soft, porous	Softer, a litte wet
	Odor	Raw material odor	Slight acid, aromatic	Acid, slight alcoholic	Strong sour, alcoholic
	Mold	-	-	-	-
	pH	5.30	5.11	4.88	4.65
CT3	Color	Gray-brown	Dark gray-brown	Dark gray-brown	Dark brown
	Texture	Soft, porous	Soft, porous	Soft, slight clumpy	Clumpy, wet, slimy
	Odor	Slight ammonia, raw material	Slight acid, slight ammonia	Slight acid, slight ammonia	Slight ammonia, vinegar
	Mold	-	-	-	-
	pH	6.20	5.68	5.24	4.94

The reduction in pH after 5 days of fermentation creates an acidic environment for the feed, which enhances the breakdown of fiber in CSB through acid hydrolysis. Organic acids such as acetic acid and lactic acid formed during fermentation break down glucosidic bonds in cellulose and hemicellulose molecules, facilitating easier fiber digestion. Additionally, the acidic environment of fermented CSB can improve the solubility of minerals (Ca, P, Mg), making them more accessible for absorption by livestock

4. CONCLUSION

The CP content of CSB was significantly improved by using solid-state fermentation with *S. cerevisiae* and *Sac. fibuligera* after 5 days of fermentation (6.61% compared to 2.77% in the control group). The highest protein content was observed when the yeast cells peaked at 282.67 million cells/g (TN1). The application of fiber-degrading enzymes and fermentation with yeast increased the CP and TP content of CSB to 20.58 and 7.24% DM, respectively. Two levels of urea supplementation in CT1 and CT2 improved the protein content of CSB. The CP and TP contents of bagasse reached 17.16 and 8.45% with 21.7 g/kg urea, and 23.56 and 8.27% with 32.6 g/kg urea. The best protein enrichment performance of CSB were achieved when the bagasse was treated with fiber-degrading enzymes, supplemented with 21.7-32.6 g/kg urea, and fermented with *S. cerevisiae* and *Sac. fibuligera* for 5 days.

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EFFECT OF YEAST FERMENTATION ON CHEMICAL COMPOSITION OF SORGHUM GRAINS

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ABSTRACT

This study investigated changes in chemical composition of sorghum (*Sorghum bicolor*) grains during 72h anaerobic fermentation with baker's yeast *Saccharomyces cerevisiae*. The ground substrate was mixed with distilled water to make different levels of moisture content (50% and 70%). The fermentation was conducted by adding 2% of *S. cerevisiae*. Samples before and after fermentation were analyzed for proximate composition, including dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF). The results showed that sorghum fermentation was most effective after 72h. Comparing the values of raw sorghum substrate, there was significant decreases ($P<0.05$) in the ash, EE, and CF contents. A significant improvement was also observed in the protein content ($P>0.05$). In overall, the chemical composition of the fermented sorghum from treatments with either 50 or 70% moisture contents did not show a clear difference, but the one with the lower moisture content should be considered to optimize the relative time and energy consumption for drying.

Keywords: *Baker's yeast, chemical composition, fermentation, moisture content, red sorghum.*

1. INTRODUCTION

Sorghum (*Sorghum bicolor*) is ranked fifth among the most important crops after wheat, rice, corn, and barley which are likely to tolerate drought and soil toxicities compared to other cereals. Sorghum grains are staple food for humans and feed for livestock all over the world and particularly important in some arid areas of Africa and Asia (Day and Morawicki, 2018). Regarding the nutritive value, cost and availability, sorghum is the next alternative to maize in animal feed. Sorghum has higher protein but lower fat than maize does. The energy value of sorghum ranges from 90 to 100% of maize, depending on the animal species (Etuk *et al.*, 2012). However, compared to maize, sorghum grains contain lower xanthophylls levels required for egg yolk pigmentation and skin coloration for poultry. Therefore, they can completely replace maize in poultry diets without negative effects on the growth and carcass yield of broiler chickens, except the yolk colour of the layer chickens (Beyer, 2021; Beriso, 2022).

The major considerations of sorghum in the food and feed system are the lower availability of protein, starch, and minerals due to the presence of anti-nutritional factors such as tannins and phytic acid. However, fermentation has been proven to desirably modify the substrate components by reducing anti-nutrients, thus improving the functional properties and feeding value of sorghum (Mohapatra *et al.*, 2017). Fermentation of solid substrates, also known as solid-state fermentation, is a process that involves microbial growth on solid substrates in the absence or near absence of water, where the substrate serves as carbon and energy sources (Abdul and Webb, 2017; Obi, 2019). The conventional approach of using solid-state fermentation with microorganisms, typically *Saccharomyces cerevisiae* and lactic acid bacteria (LAB) is regarded as a safe and excellent biotechnological strategy for bioconversion of sorghum and other cereal grains (Yafetto *et al.*, 2022). The objective of this study was to evaluate the effects of fermentation process

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using *S. cerevisiae* on the quality changes of the red sorghum grains.

2. MATERIALS AND METHODS

2.1. Fermentation substrate and yeast

Fermentation substrate: Red *Sorghum bicolor* grains (Figure 1A) were obtained from the Green Health Import Export Co., Ltd (HCM city). The grains were ground into powder using Polymix® PX-MFC90 Lab mill (Kinematica AG, Switzerland), sieve size 300μm. The sorghum powder was mixed with distilled water to achieve relevant moisture contents of 50 and 70%.

Saccharomyces cerevisiae: Commercially dried, instant baker's yeast (Lesaffre, France) was purchased from a supermarket and stored in the laboratory at room temperature until use.

2.2. Anaerobic fermentation

Prior to fermentation, yeast cells were activated by dissolving 1g of dry yeast powder in 100ml of 10% glucose solution. The suspension was incubated for 24h at room temperature. Then the fermentation was performed anaerobically in a plastic bag at room temperature by inoculating 2% (v/w) of the inoculum suspension with yeast cell density of about 10^8CFU/ml into the substrates.

The fermented sorghum was sampled to measure the pH value and viable yeast cell concentration at a 24h interval. After that, the sample was transferred to a forced convection oven (Yamato DKN812, Japan) at 55°C to inhibit further fermentation until it dried, making fermented powder (Figure 1B).

Figure 1. Raw sorghum grains as substrate (A) and 72h-fermented sorghum powder (B)

2.3. Measurements

Changes in pH values (measured with the pH meter, Horiba PH210, Japan) and the total count of yeast cells (determined on the MRS agar media) of fermenting samples were monitored for every 24h till 72h of the fermentation. The proximate compositions of sorghum samples were determined following the methods of the AOAC (2005).

2.4. Statistical analysis

Experimental data were statistically analyzed using the GLM of Minitab 16 software. Means were compared using Tukey's test at a 5% significance level ($P<0.05$).

3. RESULTS AND DISCUSSION

The results of chemical composition analysis of sorghum grains were as follows: 87.75±0.72% DM, 2.49±0.15% ash, 10.40±0.39% CP, 3.03±0.08% EE and 4.90±0.26% CF. The results were similar to those reported by Nemukondeni *et al. (*2022).

Figures 2 and 3 show the results of measured pH values and the number of yeast cells counted over 24h intervals of fermentation process. The pH values reduced from 7.22 to 4.99 and from 6.81 to 4.85, respectively in 50 and 70% moisture levels (Figure 2). An opposite trend was observed in Figure 3 in the yeast population. The number of yeast cells increased from 1.31×10^7CFU/g to 10.85×10^7CFU/g and from 1.26×10^7CFU/g to 10.49×10^7CFU/g, respectively in 50 and 70% moisture levels. These results were possibly due to the accelerated growth accompanied with the metabolic activity of yeast cells, as previously reported by Ojokoh and Eromosele (2015).

Due to the maximum number of yeast cell density reached after fermentation, either at 50 or 70% moisture levels, samples obtained after 72h fermentation were selected to assess the differences in the chemical composition with the substrate. The variables of the substrate (raw sorghum grains) as well as the fermented sorghum samples are

presented in table 1, indicating that the solid-state fermentation with *S. cerevisiae* significantly increased the CP but reduced the ash, EE and CF contents (P<0.05). Interestingly, there were non-significant differences between the values of fermented samples (P>0.05). However, considering the longer time associated with the higher energy consumption for drying, the 50% moisture level of initial substrate should be recommended.

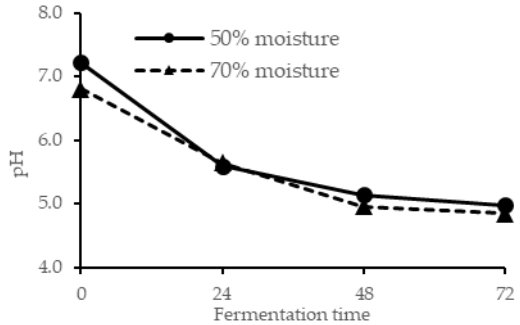

Figure 2. Changes of pH values during 72h sorghum fermentation

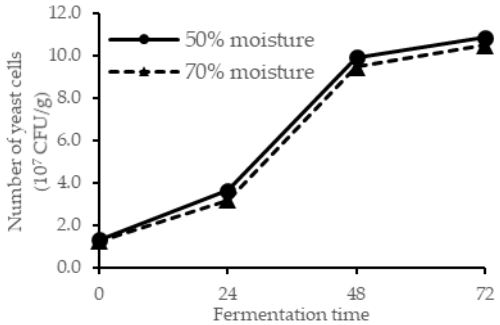

Figure 3. Changes of the number of yeast cells during 72h sorghum fermentation

Table 1. Chemical composition SG 72h fermentation

%	Sorghum substrate	Fermented sorghum		SEM	P
		50%moisture	70%moisture		
DM	89.75	88.13	86.21	1.43	0.280
Ash	2.49[a]	1.16[b]	1.28[b]	0.17	0.001
CP	10.40[b]	14.48[a]	14.01[a]	0.29	0.000
EE	3.03[a]	1.79[b]	1.15[b]	0.24	0.001
CF	4.90[a]	2.37[b]	2.00[c]	0.10	0.000

Note: Means within a row followed by different superscripts are significantly different at 5% level (P<0.05).

Percentage changes of nutrients before and after fermentation are considered as indicators of yeast ability. Figure 2 shows the changes in the chemical composition of fermented sorghum compared to the raw substrate of sorghum grains. There were decreases in the contents of DM, ash, EE, CF and an increase in the CP content after 72h fermentation. However, there was only one significant (P<0.05) change between two moisture levels which were showed in the either extract (or crude fat) content.

As can be seen in figure 4, most of the nutrients in chemical composition were reduced with fermentation, except for the CP. The nutrient loss could be due to the leaching of some substances into the aqueous medium. The current significant increase in the CP content of fermented sorghum may be due to the elevated level of amino acids during the fermentation process, which is in agreement with other studies (Day and Morawicki, 2018; Yafetto *et al.*, 2022). Similar to our results, an experiment performed with baker's yeast and sorghum grain also found that the weight loss correlated with increasing amounts of protein in the substrate (Day and Morawicki, 2018). According to Sheikh *et al*. (2024), solid-state fermentation has efficiently improved the production of secondary metabolites or value-added products, including organic compounds.

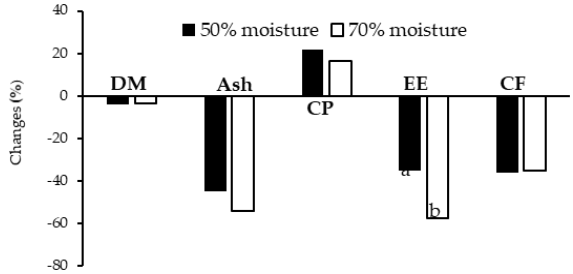

Figure 4. Chemical composition of fermented sorghum compared to the raw substrate

Note: Bars with different letters are significantly different at 5% level (P<0.05).

3. CONCLUSION

Yeast fermentation at 50 and 70% moisture levels for 72h significantly reduced

the ash, fat, and fiber but improved the protein contents of the red sorghum. Future studies should explore the amino acid profiles and bioavailability of fermented sorghum to maximize their use in animal nutrition.

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IMPROVING NUTRITIONAL VALUE OF CASSAVA AND SWEET POTATO ROOTS BY YEAST FERMENTATION

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ABSTRACT

This study investigated the changes in chemical composition of cassava and sweet potato flours during 72h of solid-state fermentation process using baker's yeast *Saccharomyces cerevisiae* as fermentation factor. Both cassava and sweet potato flours were mixed with distilled water to reach 50% moisture content, before being inoculated with 2% of activated yeast solution in solid-state fermentation conditions for 72h at room temperature. Fermented samples were taken at 24h intervals to measure the changes in pH values and yeast population. Samples were then dried at 55^{o}C in a convection oven and the products were analyzed for proximate composition. The results showed a reduction in the pH values and an increase in the microbial count during fermentation, particularly within 48h from starting point. The highest number of yeast cells was observed at 48h (4.79 and 5.06 10^{7}CFU/g, respectively in the fermented cassava and sweet potato), leading to an efficient increase in the protein and reduction in the fiber contents of the two substrates. Particularly, remarkable changes in protein and fiber levels were higher in the cassava samples compared to the sweet potato samples. Therefore, the cassava fermented product, harvested at 48h of the fermentation has the potential to be used as functional feed additives in animal diets.

Keywords: *Baker's yeast, chemical composition, fermentation, cassava root, sweet potato root.*

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) are ranked in the top five tropical root and tuber crops produced and consumed worldwide. They are destined mainly for human consumption, animal feed or industrial processing, notably in the manufacturing of starch, alcohol, and fermented beverages (OECD/FAO, 2023). In Vietnam, these traditional staples are particularly considered as pivotal crops in the 21st century, not only due to their nutritive and caloric values, but also their adaptability to diverse growing conditions. According to the vision of the Vietnamese Government and the Ministry of Agriculture and Rural Development, root and tuber crops alongside cereal crops, occupies a primacy position in research and development of the food and feed sector (Hoang *et al*., 2024).

Nutritionally, cassava and sweet potato have a great potential to provide dietary energy sources (mainly in carbohydrates), equivalent to one-third of the energy level provided by rice or wheat. In addition, their outstanding biomass and cumulative root yield supply more energy per land unit per day compared to cereal grains (Truong *et al*., 2018; Chandrasekara, 2019). In spite of multi-beneficial nutrients, such as carbohydrates, vitamins, minerals, dietary fiber, proteins and essential amino acids, these roots also contain anti-nutritional factors and toxic substances, which critically impair animal uptake and absorption of nutrients. While cassava has phytate, nitrate, polyphenols, oxalate and saponins; sweet potato also has raffinose and protease inhibitors which generally reduce nutrient bioavailability (Ajayi *et al*., 2016; Zekarias *et al*., 2019). However, proper processing approaches are

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able to detoxify or reduce the impact of antinutrients and improve nutritional value of these tropical root crops. Among them, fermentation is an effective and sustainable processing method that increases the shelf-life as well as physico-chemical composition of the substrates (Yuliana *et al.*, 2017; Hidayat *et al*., 2020; Maicas, 2020).

To our knowledge, information about the fermentation process conducted by baker's yeast (*Saccharomyces cerevisiae*) on the quality of tropical root crops have not been fully understood. Hence, this study was designed to investigate the influence of yeast fermentation on the nutritional changes of cassava and sweet potato roots, to provide novel techniques for feed processing and quality enhancement.

2. MATERIALS AND METHODS

2.1. Substrates and yeast inoculum

Substrates: Roots of cassava and white-fleshed sweet potato were purchased from a local market in Dong Nai province. The roots were washed with tap water to remove dirt and soil before peeling. During the process, they were kept in tap water to prevent enzymatic darkening. Peeled roots were cut into slices (1-2mm thickness) using a hand peeler. The slices were then sun-dried for 2 days. The dried slices were ground using the Polymix® PX-MFC 90 Lab mill (Kinematica AG, Switzerland), and sieved through a 300µm-mesh screen to obtain cassava and sweet potato flours. The flours were mixed with distilled water to achieve relevant moisture contents of 50%.

Saccharomyces cerevisiae: Commercially instant baker's yeast (Lesaffre, France) was purchased from a supermarket in and stored in the laboratory at room temperature. The yeast cells were activated by dissolving 1g of dry yeast powder in 100ml of 10% glucose solution. The suspension was incubated for 24h at room temperature.

2.2. Anaerobic fermentation

The yeast inoculum with cell density of about 10^8CFU/ml was mixed with the substrates (cassava and sweet potato flours) at a rate of 2% (v/w). The mixtures were allowed to ferment for 72h at room temperature. Samples were collected at 24h interval to measure the pH values and yeast cell concentration. The samples were then dried in a cabinet oven (Yamato DKN812, Japan) at 55°C, making fermented cassava and sweet potato flours.

2.3. Measurements

The pH values of the fermented samples were determined using a pH meter (Horiba PH210, Japan) which has been previously adjusted with buffer solutions of pH 4 and 7. Total count of yeast cells (determined on the MRS agar media) of the fermenting samples were monitored for every 24 till 72h of the fermentation. All analysis for proximate compositions in both substrates (cassava and sweet potato flours) as well as fermented samples was carried out in triplicates by the methods described by AOAC (2005).

2.4. Statistical analysis

All data obtained from the ANOVA using the GLM of Minitab 16 software. Differences of the Means were reported at a significance level of 0.05 ($P<0.05$).

3. RESULTS AND DISCUSSION

Table 1 shows the proximate compositions of the cassava and sweet potato flours as substrates of the fermentation trials. There were non-significant differences ($P>0.05$) between two samples in terms of DM, ash, protein, fat and fiber. The results were similar to those obtained previously by other authors (Titus and Lawrence, 2015; Lareo *et al*., 2013).

Table 1. Chemical composition of substrates (%)

Composition	Cassava	Sweet potato	SEM	P
DM	88.67	88.89	0.25	0.564
Ash	2.22	1.72	0.13	0.052
Protein	2.11	2.35	0.09	0.146
Fat	0.67	0.69	0.05	0.769
Fiber	2.96	2.91	0.11	0.764

It is important to determine pH values as relatively low pH inhibit pathogen

development and spoilage which increase the shelf life of fermented products. The results of the pH values and yeast count measured in fermented samples are presented in figure 1. Significant changes (P<0.05) in pH among the time intervals either in the fermented cassava or the fermented sweet potato samples, which decrease from pH 4-6 to 3-4 were observed at the beginning and final of the fermentation process. In general, the pH levels of the cassava samples were always higher than those of the sweet potato samples.

Inverse trends were observed between the pH values and the number of yeast cells over time. The total population of yeast cells rose with fermentation time and differed non-significantly (P>0.05) between the substrates. It is noteworthy that a continuous increase in the yeast population was observed after 2 days of fermentation. However, a decrease in the yield of ethanol was noticed on the third day of fermentation. The microbial count as shown in this study was in line with earlier works by Nguyen *et al*. (2024), who indicated that numbers of yeast cells increased during 48h and slightly reduced at the later stage of rice by-products fermentation. According to Ajayi *et al. (*2016), metabolites such as organic acids, phenolic compounds, esters and carbon dioxide play an important role in lowering the pH and increasing acidic levels of the fermented products.

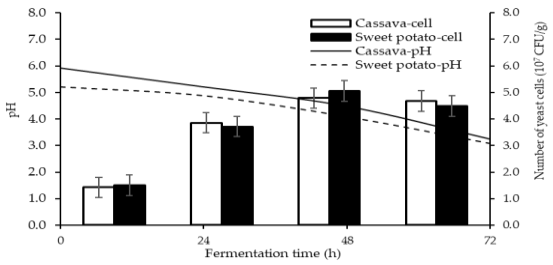

Figure 1. Changes of pH values and yeast growth during 72h fermentation

Note: Error bars represent standard errors of the mean (SEM)

The experimental data revealed that the fermentation period of 48h yielded the highest amount of yeast cell production, being 4.79 and 5.06 10^7CFU/g, respectively in the fermented cassava and sweet potato. Therefore, samples obtained at 48h fermentation were used to compare the chemical composition as well as changes in the constituents between the two substrates.

As can be seen in table 2, despite the non-significant observed in the proximate composition between fermented cassava and fermented sweet potato samples, there were substantial increases in DM and protein but decreases in ash, fat and fiber contents of the fermenting either cassava or sweet potato flours compared to the samples before fermentation. However, the significant differences (P<0.05) in percentage changes between two substrates were observed only in protein and fiber contents. The remarkable changes in protein and fiber of the cassava samples (125.28 and -58.88%, respectively) were higher than those of the sweet potato samples (93.61 and -38.83%, respectively).

Table 2. Chemical composition after 48h fermentation

Variables		Cassava	Sweet potato	SEM	P
Composition (%)	DM	91.21	90.54	0.64	0.472
	Ash	1.55	0.93	0.20	0.052
	CP	4.75	4.54	0.11	0.222
	Fat	0.39	0.45	0.11	0.717
	Fiber	1.49	1.53	0.13	0.817
Changes (%)	DM	2.87	1.86	0.72	0.340
	Ash	-30.08	-45.74	11.07	0.341
	CP	125.28^a	93.61^b	5.16	0.001
	Fat	-41.54	-34.78	16.11	0.773
	Fiber	-58.88^b	-38.83^a	3.86	0.004

Notes: Values in the same row with the same superscript are not significantly different (P<0.05).

According to Hidayat *et al.* (2020), the fermentation process opens up the fiber structure, so resistant starch and other dietary fiber becomes more available for microbial activity. In addition, during fermentation, *S. cerevisiae* cells produced extracellular enzymes, such as amylases and cellulase that decomposed starch and dietary

fiber as sources of carbon for its growth. The multiplication of the yeast cells as a source of single cell protein could also provide an explanation for the increase in protein content of fermented cassava and sweet potato products. Thess protein-enriched products after fermentation could be used as a protein source in animal feed to provide economically viable and sustainable development of livestock production.

3. CONCLUSIONS

The solid-state fermentation using cassava and sweet potato flours as substrates and baker's yeast *S. cerevisiae* as fermentation factor efficiently increased the protein and reduced fiber contents of the two substrates. The remarkable percentage of increased protein and decreased fiber levels were higher the cassava samples compared to the sweet potato samples. Therefore, the cassava fermented product, harvested at 48h of the fermentation has the potential to be used as functional feed additives in animal diets.

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PRESERVATION OF TOTAL MIX RATION (TMR) FOR BEEF CATTLE IN SUMMER

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ABSTRACT

The objective of this study was to evaluate effects of chemical and biological additives on quality and prolongation of storage time of TMR diets for beef cattle in summer. TMR experimental diet was mixed by king grass, whole corn silages, rice straws, cassava residues, wheat brans, soybean meal, brewery wastes, minerals and vitamines. The TMR diet was formulated according to recommendation of NRC (2016) to meet the nutrient requirements BBBxZebu cattle from 19-21 months of age. TMR diets were divided into 4 treatments (three bags/treatment, 25kg/bag): (1) no additives (control-ĐC); (2) TMR+potassium sorbate (1g/kg FM) (PS); (3) TMR+Sodium benzoate (1g/1kgFM) (SB); (4) TMR+a mixture of propionic acid (0.3%) and *L. plantarum* inoculant ($1x10^6$cfu/ml) (PL). DM, CP, NH_3-N, organic acid content, pH and microbial counts were evaluated at days 1, 3, 5, 7, 9, 12, 15 of preservation. The results showed that supplementing of propionic acid and *L. plantarum* into TMR diets after mixing obviously maintained stably TMR quality and prolonged storage time until 9 days to compare with other treatments. PL formular is recommended as additive to maintain TMR quality and lengthen storage time.

Keywords: *Preservation, TMR, beef cattle, organic acid salts, propionic acid, Lactobacillus plantarum.*

1. INTRODUCTION

Total Mixed Ration (TMR) is currently being widely used in Vietnam's cattle farming because it meets the nutritional requirements of cattle. However, TMR diets have short storage shelf life because it is easy to spoil when being exposed to air or feed-out in summer. Exposure to air can stimulate the activity of yeasts and aerobic bacteria. These undesirable microorganisms consume soluble carbohydrates in feed to produce unwanted products such as ammonia, butyric acid, CO_2 and water, leading to the decrease of lactic acid content, increase of TMR pH, and raising TMR temperature. In the cattle barns, secondary fermentation can occur after just a few hours when TMR diets are feed-outed to cattle. This fermentation process in the feed generates heat, raising the temperature of the TMR, leading to quick spoilage, reduced feed quality and feed intake. Therefore, in order to maintain TMR quality and prolong preservation time, it is essential to seek effective methods to reduce undesirable microbial growth, heat generation and losses through aerobic spoilage. Extending the storage time and utilization of TMR will help to reduce mixing labor and make the TMR product easier to market.

Biological and chemical additives are good choices to maintain lower pH of TMR and inhibit the proliferation of aerobic microorganisms during mixing and feeding times. Chemical additives mainly focus on some organic acid salts such as sodium benzoate (SB) and potassium sorbate (PS) can ionise to produce organic acids and salt ions, which have acidic characteristics and antibacterial property (Dai *et al.*, 2022). Lactic acid bacteria (LAB) were utilized to decrease the biogenic amine production and improve the aerobic stability of silage (Nishino *et al.*, 2007). This study aimed to evaluate the effectiveness of using organic acid salts and LAB additives on preservation of TMR in the summer period.

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2. MATERIAL AND METHODS

TMR formula: The TMR diet was formulated according to National Research Council (NRC) (2016) to meet the nutrient requirements for BBBxZebu cattle of 19-21 months of age (Table 1).

Preservation additives: Four treatments were individually prepared as follows: (1) deionized water alone (Control); (2) Potassium sorbate (PS- purity 98%, from Nippon Gohshei, Yaesu, Chuo-ku, Tokyo, Japan) applied at 1g/kg FW; (3) Sodium benzoate (SB-purity 99%, from Emerald Kalama Chemical, Kalama Washington USA) applied at 1g/kg FM; (4) The mixture of homofermentative *Lactobacillus plantarum* (PL) inoculant, applied at 1×10^6 colony-forming units (cfu)/g FM and Propionic acid (PL, purity 99%, from Guanghua Sci-Tech Co., Ltd., Guangdong, China) applied at 0.3% of fresh matter (FM). Inoculants were made and enumerated in de Man, Rogosa and Sharp (MRS) broth (HiMedia Laboratories LLC, India) to determine inoculation rate of *L. plantarum*.

Table 1. TMR formula for 19-21 months of age

Ingredients (%FM-fresh matter)	TMR	Chemical components	
Elephant grass	40.14	DM, %	39.55
Whole-plant corn silage	19.54	ME, MJ/kgDM	10.78
Dry rice straws	3.07	CP, % DM	13.15
Cassave gabages	2.63	CF, % DM	15.70
Wheat brans	3.51	Ca, % DM	0.36
Cassava root meals	8.40	P, % DM	0.29
Soybean meals	4.39		
Brewery wastes	13.06		
Molasses	4.99		
DCP	0.21		
Vitamin-mineral premix	0.06		
Total	100		

Table 2. Experiment design for TMR preservation

Treatments	Summer Diet
ĐC	TMR1 (non-preservation additives)
PS	TMR1+Potassium sorbate (1g/kg FM
SB	TMR1+Sodium benzoate (1g/kg FM
PL	TMR1+Propionic acid, 0,3% + *Lactobacillus plantarum,* $1x10^6$cfu/g FM

Preservation methods: Propionic acid was diluted with deionized water to an equivalent of 0.3% FM and mixed with 10ml/kg FM of *L. plantarum*, then 4ml of solution/kg FW was sprayed into the TMR. Two types of organic acid salts are also diluted with deionized water to a ratio of 1g/1kg of fresh weight and 5ml of solution/kg FM was applied using a hand sprayer. An equal volume of deionized water was applied to the control TMR (Chen *et al.,* 2016). All the TMR diets and additives were constantly mixing by feed mixer and divided into 3 plastic per each treatment (25kg/bag). All of 12 bags were not sealed and maintained at room temperature for 15 days.

***Measurement of sampling and analytical procedures*:** 500g sample of each treatment was collected according to TCVN 13052:2021 at 1, 3, 5, 7, 9, 12 and 15 days of preservation. Temperature of TMR and preservation environment was measured using probe thermometer (TFA, AT-1018, Germany) three times daily at 8am, 12pm, 4pm. The organoleptic test was carried out by including color, odor, texture, presence of fungi, pH, and was evaluated by scoring with 1-3 scores on each parameter (Tahuk *et al.*, 2020). The results were calculated by averaging all score of 4 parameters and comparing among treatments.

Table 3. Scoring of the physical characteristics of TMR during preservation period

Parameters	**1** (bad quality)	**2** (medium)	**3** (good)
Color	Dark brown or black	Brownish yellow or brown	Yellowish green, Yellowish-brown
Odor	Burnt, ammonia, tobacco, rancid/putrid	Strong acid or alcohol	little odor or slightly acid smell
Texture	Clumping, Slimy, flabby	Slightly soft, a bit wet, clumpy	Firm, softer; Not clumpy, not slimy
Presence of fungi	Mold in the surface (<10cm) and other parts	White mold in the surface (<5cm)	None/slight white mold in the surface (<1cm)

The pH was determined using a pH meter (Mettler Toledo, Switzerland). The total organic acid and alcohol concentrations were measured by refractometer (Antago Co. LTD, Japan). Dry matter DM, crude protein (CP), NH_3-N were analyzed according to Vietnamese Standard system (TCVN 4326-2007; TCVN 4328-2007; TCVN 10494: 2014). Enumeration of LAB was performed by the pour plate technique using MRS agar. Yeasts were counted on spread plates of malt extract agar (Chen *et al.,* 2016). The numbers of aerobic microbes were determined on Plate Count Agar-medium (HiMedia Laboratories LLC, India) at 30°C/24-48h.

***Statistical analyses*:** The statistical procedures were conducted by R4.2.2 (Team, 2022). Data on organoleptic test scores were analyzed by Kruskal test and Pairwise comparisons using Wilcoxon rank sum test. The other data on preservation of TMR were analyzed by one-way ANOVA. Statistical differences among means were determined using Tukey's multiple comparison. Values of P<0.05 were regarded as significant.

3. RESULTS

3.1. Quality of TMR diets during preservation

The physical characteristics of TMR is shown in table 4. PL treatment had highest (P<005) organoleptic test scores among all treatments. The addition of propionic acid and *L. plantarum* is an effective method to maintain TMR quality stable at high temperatures. There was no change in physical characteristics of TMR added after 5 days of preservation. Slight white mold on the surface could be seen in all bags of PL treatment on day 12.

Table 4. Effects of preservation additives on physical characteristics of TMR

Days of preservation	Treatments				SEM	P
	ĐC	PS	SB	PL		
1	3.00	3.00	3.00	3.00	0.00	-
3	2.58^{bc}	2.83^{ab}	2.92^{ab}	3.00^{a}	0.09	<0.05
5	2.25^{b}	2.75^{a}	2.83^{a}	3.00^{a}	0.16	<0.001
7	2.00^{c}	2.42^{b}	2.58^{b}	2.92^{a}	0.19	<0.001
9	1.50^{c}	2.17^{b}	2.25^{b}	2.58^{a}	0.23	<0.001
12	1.33^{c}	2.08^{b}	2.17^{b}	2.42^{a}	0.23	<0.001
15	1.08^{c}	1.75^{b}	1.83^{b}	2.25^{a}	0.24	<0.001

Values in the same row with different following letters are significantly different (P<0.05)

3.2. Changing of TMR's temperature during preservation

The average room temperature during the experiment period in July 2024 ranged from 30 to 34, highest on days 5 and 7. Under high storage temperature, the lowest feed temperature (<30°C) was only recorded in the PL treatment throughout the storage period (Figure 1).

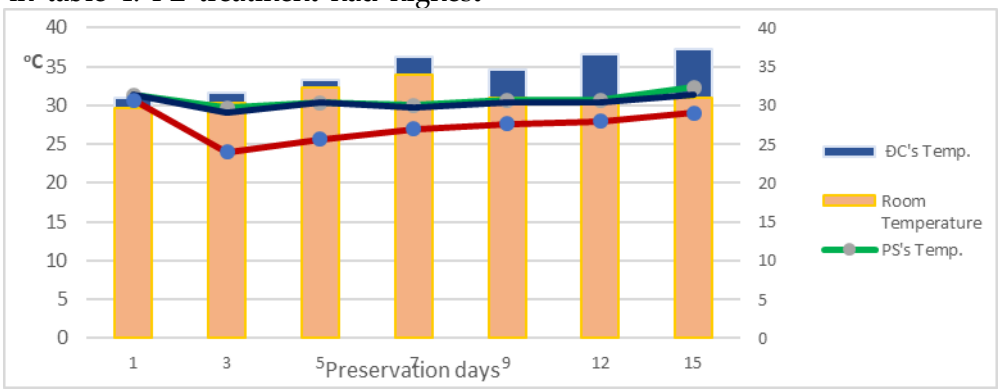

Figure 1. The change in TMR temperature to compare with storage room temperature

3.3. Chemical and microbial compositions of TMR during preservation

The changes in chemical and microbial compositions are shown in Table 5. DM reduced significantly (P<0.05) in ĐC treatment at day 7 of preservation compared with other treatments. There was no significant change in DM and CP contents of

PS, SB and PL treatments throughout the preservation period. Concentration of NH_3-N increased rapidly in ĐC treatment after 3 days of preservation, from 83,25 to 95.89 g/kg TN, and reach >100g/kg TN from day 5 to day 15. The NH_3-N in silage is an indicator of the degree of protein degradation. In well-preserved silage, the NH_3-N content should not exceed 100 g/kg total N (McDonald *et al.*, 2002). The PL treatment was the only group in which the NH_3-N content was consistently maintained below 90g/kgTN during the 15-day preservation period.

Table 5. Effects of preservation additives on chemical and microbial compositions during preservation

Items	Tr	Days of preservation						
		1	3	5	7	9	12	15
DM (%FM)	ĐC	39.56	39.47	39.23	39.06	38.58^{b}	37.80^{b}	37.26^{c}
	PS	39.54	39.51	39.43	39.33	39.34^{a}	39.19^{a}	38.44^{b}
	SB	39.57	39.52	39.45	39.38	39.35^{a}	39.25^{a}	38.53^{b}
	PL	39.56	39.55	39.55	39.48	39.41^{a}	39.33^{a}	39.27^{a}
CP (%DM)	ĐC	13.16	13.05	12.95	12.19^{b}	11.77^{b}	11.15^{b}	10.55^{b}
	PS	13.18	13.16	13.17	13.10^{a}	13.06^{a}	12.85^{a}	12.66^{a}
	SB	13.16	13.17	13.15	13.11^{a}	13.08^{a}	12.89^{a}	12.75^{a}
	PL	13.17	13.22	13.25	13.18^{a}	13.16^{a}	13.13^{a}	13.06^{a}
NH_3-N (g/kg tota N)	ĐC	83.25	95.89^{c}	101.54^{c}	106.2^{c}	115.41^{c}	134.50^{c}	150.06^{c}
	PS	82.66	87.92^{b}	86.05^{b}	88.12^{b}	90.18^{b}	99.61^{b}	108.78^{b}
	SB	82.65	85.03^{ab}	84.67^{b}	87.64^{b}	92.91^{b}	97.87^{b}	105.33^{b}
	PL	82.47	81.30^{a}	80.24^{a}	81.90^{a}	82.34^{a}	83.61^{a}	86.87^{a}
pH	ĐC	5.54	5.77^{b}	5.86^{b}	5.74^{b}	5.84^{c}	6.18^{c}	6.76^{c}
	PS	5.25	4.49^{a}	4.58^{a}	4.77^{a}	5.01^{b}	5.11^{b}	5.56^{b}
	SB	5.21	4.47^{a}	4.59^{a}	4.65^{a}	4.94^{b}	5.13^{b}	5.57^{b}
	PL	5.14	4.34^{a}	4.15^{a}	4.38^{a}	4.41^{a}	4.53^{a}	4.79^{a}
Organic acid (% FM)	ĐC	1.15^{b}	0.94^{c}	0.74^{c}	0.55^{c}	0.38^{c}	0.21^{c}	0.19^{c}
	PS	1.71^{a}	1.85^{b}	1.58^{b}	1.24^{b}	1.13^{b}	1.07^{b}	1.04^{b}
	SB	1.72^{a}	1.84^{b}	1.65^{b}	1.33^{b}	1.12^{b}	1.11^{b}	1.08^{b}
	PL	1.85^{a}	2.27^{a}	2.22^{a}	2.24^{a}	2.16^{a}	1.73^{a}	1.75^{a}
LAB (log cfu/g FM)	ĐC	5.04^{b}	4.58^{c}	4.18^{c}	3.36^{c}	3.05^{d}	2.83^{c}	2.27^{c}
	PS	5.41^{a}	5.85^{b}	6.25^{b}	5.98^{b}	5.34^{c}	4.87^{b}	3.91^{b}
	SB	5.46^{a}	5.89^{b}	6.23^{b}	6.05^{b}	5.67^{b}	4.89^{b}	3.97^{b}
	PL	5.61^{a}	6.67^{a}	7.17^{a}	6.80^{a}	6.68^{a}	6.09^{a}	5.37^{a}
Aerobic bacteria (log cfu/g FM)	ĐC	2.37	3.57^{c}	5.46^{b}	6.15^{c}	6.31^{c}	7.02^{c}	7.31^{c}
	PS	2.09	1.88^{b}	1.74^{a}	1.93^{b}	2.70^{b}	4.15^{b}	5.01^{b}
	SB	2.13	1.71^{ab}	1.68^{a}	1.82^{b}	2.76^{b}	4.21^{b}	5.06^{b}
	PL	2.04	1.26^{a}	1.29^{a}	1.34^{a}	1.43^{a}	2.35^{a}	2.83^{a}
Yeasts (log cfu/g FM)	ĐC	1.36	2.90^{b}	3.62^{b}	4.34^{c}	5.32^{c}	5.17^{c}	5.40^{c}
	PS	1.20	1.16^{a}	1.12^{a}	1.54^{b}	2.15^{b}	3.46^{b}	4.13^{b}
	SB	1.21	1.12^{a}	1.13^{a}	1.49^{b}	2.11^{b}	3.33^{b}	4.07^{b}
	PL	1.16	1.10^{a}	1.04^{a}	1.06^{a}	1.04^{a}	1.14^{a}	2.45^{a}

Values in the same column with different following letters are significantly different (P<0.05)

The pH value of ĐC treatment increased continuously (from 5.54 to 6.76) throughout experiment. In contrast, a decline in pH value during storage was observed in three groups using preservatives PS, SB and PL. However, pH value maintained steady <4.50 until 9 days of preservation was only observed in PL treatment, indicating this treatment was well preserved. Besides, PL treatment had highest organic acid (OA) content and LAB population (P<0.05) among all treatments. Using organic acid salts, propionic acid and *L. plantarum* was significantly control of yeast and aerobic bacteria (P<0.05) during storage. In this experiment, the aerobic bacteria and yeast counts were lowest in PL treatment, less than 3 log_{10}cfu/g FM, indicating the aerobic stability of this TMR diets as adding the mixture of propionic acid and *L. plantarum* throughout the preservation.

4. DISCUSSION

In many beef cattle farms in hot areas of Vietnam, TMR diets can be fed in short time, event only on the day after mixing, because of several reasons (i) When feed ingredients are mixed together, it increases the pH of the diet, leading to rapidly increasing bacilli and other aerobic bacteria population, then raising feed temperature, (ii). The feed is exposed to air, creating conditions for aerobic microorganisms to grow and proliferate, initially respiring water-soluble carbohydrate (WSC) and then more complex substrates, leading to reduce TMR quality, (iii) High barn temperatures in the summer can cause fast fermentation and spoilage of the TMR. It is obviously that in the ĐC treatment (no adding of preservation additives), pH at the first day of preservation was 5.54 and increased continuously after then. This pH level is suitable for proliferation of wild yeasts and aerobic bacteria. In addition, an average room temperature of 30-34°C was suitable for growing of yeasts and aerobic bacteria such as bacilli, enterobacteria. These unwanted microorganisms induce a loss of DM due to their consuming WSC to produce

CO_2 and water, thus increasing pH and feed temperature (Carvalho *et al.,* 2014). Besides, lactate-utilizing yeasts can oxidise lactic acid into CO_2 and water, leading a raising of pH. Enterobacteria cause decarboxylation and deamination of amino acids in TMR's ingredients, thereby increasing NH_3 content and biogenic amine production. As a result, the texture of TMR changed to clumpy, wet and slimy. The odor and color of feed was changed from strong acid to ammonia or tobacco odor with brown or black color. Moreover, the population of yeast and aerobic bacteria of ĐC treatment was exceed 4 log cfu/g FM, indicating a higher aerobic deteriotaion after exposure to air during preservation (lowest organoleptic test scores; lowest organic acid and highest NH_3-N content).

In this study, potassium sorbate, sodium benzoate, propionic acid, and *L. plantarum* (PL treatment) were added to TMR diets after mixing to control temperature rising and undesirable microorganisms during TMR's preservation. The mixture of propionic acid with a homogeneous *L. plantarum* was to improve effectiveness of controlling undesirable microorganisms because homogeneous *L. plantarum* strains can produce higher quantities of lactic acid than other organic acids, leading to rapidly reduce of feed pH. Propionic acid is a potential antimicrobial agent (Dai et al., 2022). When applied at a concentration of 0.3% FM, it has been shown to effectively inhibit the activity of unwanted microbes. This results in lower populations of aerobic bacteria and yeasts, minimizing aerobic deterioration and reducing NH_3-N content. LAB had the highest proliferation in PL treatment at day 3 and remained high number until day 9 of storage. This result can be explained by two following reasons: (i) the aerobic bacteria's consumption oxygen entering the TMR at the surface exposed, creating anaerobic conditions favorable for LAB growth; (ii) The inhibition of aerobic microorganisms due to low pH can reduce the competition for resources between LAB and harmful microorganisms.

Previous studies found that potassium sorbate and potassium sorbate were effective in inhibiting yeasts, molds, and spore-forming bacteria in corn silage because it can ionise to produce sorbic acid, benzoic acid and salt ions (Kung et al. ,2018). Dai et al., (2022) reported that SB and PS were more effective to improve aerobic stability of fermented TMR than untreated TMR. In the present study, PL treatment was more stable in maintaining low pH, aerobic microorganism population, NH_3-N content during 15 days of preservation compared to PS and SB treatments. By comprehensive consideration, PL is recommended as additive to maintain TMR quality and lengthen storage time.

5. CONCLUSIONS

The results suggested that supplementing of propionic acid and lactic acid bacteria into TMR diets after mixing obviously maintained stably TMR quality and prolonged storage time until 9 days to compare with PS and SB treatments, as indicated by higher organic acid content, LAB counts, lower pH, NH_3-N contents, aerobic bacteria and yeast counts. PL formular could be an effective solution to maintain TMR quality and extend the time of storage and utilization of TMR in summer time.

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FRUIT BY-PRODUCTS USED AS PELLET INGREDIENTS FOR SUSTAINABLE RUMINANT FEEDING: NUTRITIONAL PROPERTIES

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ABSTRACT

The objective of this study was to investigate the potential use of agricultural wastes consisting of pineapple (*Ananas comosus*) peels, passion fruit (*Passoflora edulis*) peels, sweet corn husk and corn cob (*Zea mays L. var. rugosa Bonaf.*), banana (*musa*) peels and pellets from fruit peels (PPM and PPT) as ruminant feeds and enteric methane mitigation. The samples were analyzed for proximate including dry matter (DM), crude protein (CP), Ash, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), total sugar; and phytonutrient including tannins and saponins. The study highlighted that pineapple, passion fruit, mangosteen peels and corn husks and cobs with low to moderate CP, higher CF contents could be use as fiber sources for ruminants while banana peels with moderate CP and low CF levels could be used as energy sources for ruminants. The PPM and PPT pellets had moderate CP and higher CF contents and especially contained 1.8-4.2% tannins and 8.2-11.02% saponins could be considered as potential fiber feed sources for the purpose of improving rumen pH, fermentation, nutrient digestibility and methane mitigation.

Keywords: *By-products, pellets, chemical composition, ruminants.*

1. INTRODUCTION

Currently, the world has over 8 billion people and by 2050 this number is expected to increase to 9 billion, this increase and concerns about food security are a huge challenge for land use. Annual meat consumption is expected to increase 70%. The increasing future demand for livestock products will impose a huge demand for feed resources. "Feed–food competition" was defined the tensions and trade offs between two alternatives uses for edible crops: direct consumption by humans versus feeding livestock (Breewood and Garnett, 2020). Therefore, a key to sustainable livestock development is efficient use of available feed resources including wastage, and enlargement of the feed resource base through a quest for novel feed resources, particularly those not competing with human food. Vietnam has a variety of plant resources that can be used to produce food or other products. Currently, the total amount of agricultural by-products in Vietnam is nearly 160 million tons, of which, there are about 90 million tons of post-harvest by-products from crops and from food processing of the crop sector (General Statistics Office of Vietnam, 2021).

Fruit peel is traditionally discarded in huge amounts by the food processing industry. If this by-product source is not used, it will be wasteful and cause environmental pollution. Fruit peels have a potential to be included in the ruminant diet as a rich source of protein, soluble sugars and minerals (Khattak and Rahman, 2017), especially those rich in phytonutrient (phenolics, flavonoids, saponins and tannins)

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(Branciari *et al.,* 2021). These bioactive compounds modulate ruminant microorganisms, fermentation, digestion and reduce the methane emissions (Vastolo *et al.,* 2020). The nutritional value of fruit waste as a promising alternative feed for the animals has been done in several studies (Angulo *et al.,* 2012; Matra *et al.,* 2020; Wanapat *et al.,* 2021). Supplementation of 18.0% fruit waste as part of concentrate in the diet of dairy animals produced milk with a higher proportion of health beneficial fatty acids without compromise in daily milk yield (Angulo *et al.,* 2012). Matra *et al.* (2020) reported that the addition of 400g of dragon fruit peel pellet resulted in improved rumen fermentation end products especially propionate and microbial protein synthesis. The supplementation of composite fruit peels of mangosteen, rambutan and banana improved nutrient digestibility and efficiency of microbial nitrogen supply, mitigation rumen methane production and reducing protozoal population (Wanapat *et al.,* 2021).

Feeding the ruminants with fresh fruit waste is difficult since the waste are easily rotten and cannot be stored for the long-term usage. Thus, converting the waste into pellets are the suitable method in handling such waste where the pellet form can increase the bulk density, improve the storability as well as reduce the cost of transportation. Furthermore, feed pellets are easier to control over the desired feed ration with the nutritional needs for animals (Zainuddin *et al.,* 2014).

In this study, fruit peels of pineapple, passion fruit and mangosteen and/or green tea crumbs were combined to the pellet feeds to utilize at the same time and thoroughly the source of by-products which are available on farm or at industrial canning factories. With the hypothesis that the fruit peel pellet as low production costs that contained phytonutrients would improve rumen fermentation characteristic, nutrient digestibility and methane emission. In present study, we initially evaluated the possibility of producing pellets from composited fruit peels and evaluated the chemical composition of the pellets for suitability as feed for ruminants.

2. MATERIALS AND METHODS

2.1. Raw materials

Agricultural wastes consisting of pineapple (*Ananas comosus*) peels, passion fruit (*Passoflora edulis*) peels, sweet corn husk and corn cob (*Zea mays L. var. rugosa Bonaf.*), banana (*musa)* peels in fresh form were collected from Doveco – Dong Giao Foodstuff Export Joint Stock Company, Ninh Binh province. Mangosteen (*Garcinia mangostana* L.,) peels and green tea crumbs were obtained from local markets in Trau Quy, Gia Lam, Hanoi. The wastes were stored in the plastic bags for further processing of chemical composition analysis.

2.2. Pellet preparation

Table 1. Pellet formulation of fruit peel by-product

Ingredients (%)	**PPM532**	**PPM352**	**PPT532**	**PPT352**
Pineapple peel	50	30	50	30
Passion fruit peel	30	50	30	50
Mangosteen peel	20	20		
Green tea crumbs			20	20

Note: PPM532, 352: pellet feed containing 50, 30%; pineapple peel, 30, 50%; passion fruit, 20, 20% mangosteen peel; PPT532, 352: pellet feed containing 50, 30% pineapple peel; 30, 50% passion fruit; 20, 20% green tea crumbs, respectively.

Three samples of fruit peels including pineapple, passion fruit and mangosteen peels then were chopped and sun-dried for approximately 3 days. Dried fruit peels and green tea waste were processed by griding to the length of 1mm sieve. Four formulars of pellet were produced. All the ingredients on a DM basis were well mixed with water and processed by pellet machine to make the pellet product. The percentage of ingredients in 1 kg of feed are presented in table 1. Water was added at 15%. After that, the pellet was sun dried to contain at least 850 g/kg DM. The entire process of raw material preparation and pellet production were

carried out at the Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, VNUA.

2.3. Chemical analysis

Samples of each agricultural wastes and pellets were analyzed for DM, crude protein (CP), Ash, crude fiber (CF), neutral-detergen fiber (NDF), acid-detergen fiber (ADF), ether extract (EE) by using the standard methods of Vietnam at the Center Laboratory of Faculty of Animal Science, Vietnam National University of Agriculture. DM was determined according to TCVN 4326:2001; CP according to TCVN 4328-1:2007; CF according to CN-PP.03:2019 (ANKOM Technology method 7); EE according to TCVN 4331:2001; CF according to ANKOM Technology method 6; ADF according to ANKOM Technology method 5; Ash according to TCVN 4327:2007. To analyze phytonutrient components including total tannins and saponins, samples were sent for analysis at the National Institute for Food Control where total saponin content was determined using the NIFC.05.M.182 method.

3. RESULTS AND DISCUSSION

3.1. Nutritional profile of agricultural by-products

Pineapple peel (PAP) contained high moisture (81.49%), low CP (4.23%) and fat (0.42%), greater level of CF (23.53%) and NDF (60.68%). In this study, the ash content in PAP was low (3.11%); and total sugar content was quite high (34.78%). This result indicated that pineapple peel could be serve as a valuable fiber source. Our result of chemical composition of PAP was in agreement with those in other previous studies. Hemalatha and Anbuselvi (2013) have reported the low fat and protein contents in pineapple waste. Pineapple waste contained 6-8% CP, 41.92% carbohydrates, and 54.8% NDF (López-Herrera *et al.,* 2014; Idayanti *et al.,* 2022).

Passion fruit peel (PFP) had low DM (12.20%), moderate CP content (9.55%) and high level of ash (8.26%). The CF of PFP was high (31.41%). With these nutritional properties, PFP can be used as a source of forages for ruminants. In several previous studies, PFP is accounted for approximately 45-62% of the fruit, and was rich in pectin and minerals (Alves *et al.,* 2015; Wanapat *et al.,* 2024). Regarding to CP content, different values were reported. Hiep *et al.* (2020) showed PFP had relatively high contents of CP (14.1%). Alves *et al*. (2015) and Almeida *et al*. (2019) reported that the CP content of PFP was 10.2%. However, the moderate level of CP (6.80 and 8.64%) in PFP was found by Janaina *et al*. (2015) and Oliveira *et al.* (2016), respectively. The CP compositions vary greatly due to various factors, including their horticultural or geological origin, cultivating and processing methods, and climatic conditions (Sol *et al.,* 2017).

Table 2. Nutritional profile of agriculte by-product

Items, %DM	**PAP**	**PFP**	**MGP**	**BAP**	**COH**	**COC**
DM	19.51	12.20	36.13	11.56	19.29	18.83
CP	4.23	9.55	2.70	8.14	5.86	8.26
EE	0.42	0.85	2.04	5.55	0.83	2.69
Ash	3.11	8.26	3.72	12.03	2.73	2.39
CF	23.53	31.41	21.90	9.24	28.56	21.45
NDF	60.68	-	45.35	-	-	-
ADF	27.19	-	36.94	-	-	-
Total sugar	34.78	6.65	-	39.25	0.68	2.12
ME (kcal/kg)	2279	2038	2482	2959	2227	2573

PAP: pineapple peel; PFP: passion fruit P; MGP: mangosteen P; BAP: banana P; COH: corn husk; COC: corn cob; -: no analysis

Mangosteen peel had 36.13% DM with a low value of CP content (2.70%) and high level of CF (21.90%). Previous studies have found the presence of several phytonutrient (tannins, saponins) in MGP, which may have potential uses as feed additives (Pothitirat *et al.,* 2009). In present study, we had not analysed tannins and saponins contents in MGP, however, in the fruit peel pellets these chemical compounds were mentioned.

Banana peels were demonstrated a good source of energy for ruminants because they have low CP (8.14%) and low CF (9.24%). In addition, BAP contained high value of ash

(12.03%) and total sugar (39.25%). Previous reports showed that BAP the CP contents of BAP ranged from 7% (Hossain *et al.*, 2015) to 10.69% (Astuti, 2015). Handayani *et al.* (2023) indicated that BAP waste contained 6.33% ash, 4.12% fat, 4.67% CP, and 18.46 CF. Wina (2001) stated that BAP waste contained organic biomass including CP, CF, minerals, and other nutrients which are needed by ruminants.

Sweet corn husk (COH) and cob (COC) are wastes from fruit-vegetable processing factories which are not utilized. These corn wastes have potential as an alternative feed for livestock. In our study, the CP contents of COH and COC were 5.86 and 8.265%, respectively. Fiber values were 28.56 and 21.45%, respectively. Ash contents were 2.73 and 2.39%, respectively. With their nutritional profile, corn cobs and husks are potentially valuable sources of roughage for ruminants (Avila-Segura *et al.*, 2011; Van *et al.*, 2011; Liu *et al.*, 2011).

In present study, several potential dietary components in agricultural by-products for ruminants exist to serve as alternates to conventional feeds. A high-energy by-product may replace grains in the diet, a high-fiber by-product can take the place of roughage, whereas a high-phytonutrient can be used as feed supplementation.

3.2. Nutritional profile of pellet feeds

Table 3. Nutritional profile of pellets (%DM)

Items	PPM532	PPM352	PPT532	PPT352
DM	89.71	90.65	88.76	89.34
CP	7.20	7.15	13.62	13.80
EE	1.22	1.71	1.81	1.30
Ash	6.14	6.55	6.82	6.86
CF	23.03	26.29	19.41	22.78
NDF	45.01	45.06	38.36	39.02
ADF	28.19	30.98	22.99	25.59
ME, kcal/kg	2711	2605	2895	2739
Tannins	1.85		4.20	
Saponin	8.20		11.02	

Chemical composition of PPM532 and PPM352 showed that they had similar nutritive value with DM, CP, NDF and Ash of about 90, 7, 45 and 7%, respectively. Tannins and saponins contents of PPM532 were 1.88 and 8.20%, respectively. Similarly, PPT532, 352 contained the levels of DM, CP, NDF and Ash around 89, 14, 39 and 7%, respectively. Tannin and saponin values of PPT532 were 4.20 and 11.02%, respectively.

When study fruit peel pellet containing 450g/kg mangosteen peel, 300g/kg rambutan peel, 150g/kg banana flower powder and 100 g/kg cassava starch the nutritive value of this pellet were 9.5% CP; 58.1% NDF and 10.5 condensed tannins and 11.7% saponins (Wanapat *et al.*, 2021). The mixture of fruit waste including papaya, pineapple and orange had CP, EE, NDF, tannin and saponin was about of 9.3, 2.3, 23.4, 3.3 and 8.5% (Sahoo *et al.*, 2021). Previous studies indicated that phytochemicals have a direct toxic effect on methanogens (condensed tannins) or protozoa (saponins) thus, have been tested as natural feed additives to decrease CH_4 production (Kamra, 2005; Patra and Saxena, 2009; Wanapat *et al.*, 2012). Condensed tannins and crude saponins have been shown to inhibit protozoa which cause the reduction of methanogens presumably by lowering the activity of protozoal associated methanogens (Guo *et al.*, 2008). Another theory is that less H_2 is produced by lower numbers of protozoa, which can be used by methanogens for CH_4 production (Morgavi *et al.*, 2010). Khoa *et al.* (2018) stated diet containing 5% green tea by-product (or diet containing 1.25% tannin) had the lowest methane emission. The combination of 1.5% cottonseed oil and 0.5% green tea tannin improved DM intake, nutrient digestibility, and reduced total methane emissions (Tran Hiep and Chu Manh Thang, 2019). Moreover, the diet with tannins can prevent ruminal nitrogen metabolism by decreasing proteolytic bacteria (Herremans *et al.*, 2020). They can reduce the ruminal bio-hydrogenation process and increase the flow of unsaturated fatty acids to the duodenum

(Abo-Donia *et al.,* 2017). Additionally, tannins, through selective activity on ruminal bacteria, can alter the process of ruminal bio-hydrogenation and increase the amount of conjugated linoleic acid in meat and milk production (Kamel *et al.,* 2017), but this effect is dosage-dependent. As a result, tannins improve the efficiency of nitrogen use by retaining more nitrogen in the body (Aboagye *et al.,* 2018; Norris *et al.,* 2019).

Thus, the PPM and PPT pellets in our study with the above nutritional composition and especially containing tannin at the level of 1.8-4.2% and saponin at the level of 8.2-11.02% are considered to be potential feed sources for the purpose of improving rumen pH, fermentation, nutrient digestibility and methane mitigation.

4. CONCLUSION

The studied agricultural by-products almost contained moderate crude protein, high fiber and ash which have the potential to serve as functional feeds. Pelleting agricultural by-products is suitable method in handling such wastes. The PPM and PPT pellets containing 1.8-4.2% of tannin and 8.2-11.02% of saponin could be considered to be potential phytonutrient feed sources for the purpose of improving rumen pH, fermentation, nutrient digestibility and methane mitigation.

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EFFECTS OF NUVIVIT K SUPPLEMENTATION IN DRINKING WATER ON THE GROWTH PERFORMANCE OF BROILERS UNDER HEAT STRESS CONDITION

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ABSTRACT

This study aimed to determine the effect of dietary vitamin A and E on the growth performance of broiler chickens under heat-stress conditions. One hundred and eighty chicks were divided into three groups with 60 birds in each group. Chickens from the control group were receiving the basal diet and water without Nuvivit K supplementation. In contrast, the birds in the treatment groups were given basal diet and water supplemented with Nuvivit K at 100 and 150ppm Nuvivit K contains a mixture of vitamins including vitamins A and E in water-dispersible form. Growth performance was determined from the day of diet change. Chicken fed with Nuvivit K at 100ppm in water achieved consistently obtained the highest final body weight, average weight gain and lowest feed conversion ratio compared to control ($P<0.05$). Vitamins did not result in any significant differences in the feed intake and water intake of the experimental birds ($P>0.05$). Based on these results supplementation of Nuvivit K at 100ppm has a beneficial effect on broiler growth performance under heat stress conditions.

Keywords: *Vitamin, heat stress, broiler, performance*.

1. INTRODUCTION

Heat stress is a condition in which animals are unable to dissipate excess heat in their bodies to the surrounding environment, thus causing in an increase in body temperature (Sugiharto, 2020). Heat stress results in compromised performance and productivity in poultry due to reduced feed intake, nutrient utilization, growth rate, egg production and quality, feed efficiency and immunity (Mangan and Siwek, 2024). Broilers under heat stress often have lower body weight and poorer feed conversion ratio (Souza *et al.*, 2016; Sesay, 2022). In additional, heat stress often causes a reduction in the antioxidant status in chicken, leading ton an increase in oxidative stress (Khan *et al.*, 2011). Heat stress is also linked with economic losses due to higher mortality of chicken (Livingston *et al.*, 2022). Heat stress also leads to heat exhaustion, organ failure, and eventually death if it is not properly managed (Tang *et al.*, 2018; Wasti *et al.*, 2020).

The adaptation of chicken to heat stress conditions is difficult and complex. When heat stress sets in, chickens respond physiologically and behaviorally seek homeostasis and restore comfort. Understanding and managing heat stress in broilers is crucial for maintaining their health and optimizing production efficiency, especially in the tropical regions. Vitamins are essential for activities such as development, growth, and metabolism of cells. Adequate supplementation of vitamins enable broiler to cope with heat stress by supporting various physiological functions, improving immune responses, and reducing oxidative stress. Vitamin C is a powerful water-soluble antioxidant that can reduces oxidative damage to cells and tissues during heat stress (Du *et al.*, 2022). Vitamin C depresses corticosterone levels, thereby reducing the overall stress in broilers (Saiz *et al.*, 2020). On the other hand, vitamin A is oil-

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soluble is involved in essential body functions including vision, differentiation of epithelial cells, growth, and reproduction. Vitamin A maintaining the integrity of mucosal tissues, which is the first line of defense against pathogens. During heat stress, it is important to maintain a strong immunological barrier (Lin *et al.*, 2002). Vitamin E, another oil-soluble vitamin, plays a role in enhancing the immune system, enabling broilers to have better resistance against diseases during heat stress thus improving growth performance (Khan *et al.*, 2015). Vitamin E also helps to protects vitamin A from oxidative breakdown, as well as enhancing the absorption of vitamin A in the animal body. The objective of this study was to evaluate the effects of water-dispersible vitamin A and E supplementation in drinking water on the performance of broilers reared under heat stress.

2. MATERIALS AND METHODS

2.1. Experimental

A total of 180 chicks were divided into three groups with 60 birds/group (10 birds per pen and 6 replication). Chickens from the control and experimental group were provided the same basal diet (Table 1).

Table 1. Nutrient content of dietary treatments

Nutrient value	Stage (day old)		
	0-14	15-28	> 28 days
ME (Kcal/kg)	3,000	3,050	3,100
Crude Protein (%)	21.0	19.0	18.0
Crude fiber (%)	4.0	4.2	5.0
Calcium (%)	0.9-1.2	0.83-0.87	0.8-1.1
Phosphorus (%)	0.8	0.75	0.7
Methionine (%)	0.45	0.4	0.38
Lysine (%)	1.1	1.05	1.0

Nuvivit K is a premix containing water-dispersible vitamin A and E in powder form (from NBC Pacific Limited Company) specially formulated for broiler drinking water applications. The drinking water in the experimental groups were supplemented with Nuvivit K 150ppm (Nuvivit 150) and Nuvivit K 100ppm (Nuvivit 100), respecitively. The control and experimental groups were subjected to heat stress condition of 35±3°C for at least 12h a day, and relative humidity approximately 80±5% for 16h a day.

Each bird was identified individually, using a numbered plastic ring at the leg and body weights (BW) were measured from the one day to 7 weeks of age with an electronic scale. The average daily gains (ADG) were calculated according to the ratio of differences between final and initial weights to the total number of days of feeding. Feed intake (FI) was also recorded for each batch. Feed conversion ratio (FCR) was calculated by dividing FI by BWG.

2.2. Statistical analysis

The data were analyzed by ANOVA as a completely random design using the Minitab 16. The experimental unit was a pen, and significance was determined at α of 0.05. Mean ± standard deviation (SD) was used to measure all the parameters.

3. RESULTS AND DISCUSSION

Heat stress is one of the most common issues in tropical regions that affecting the growth performance of poultry. Depending on the stress duration and broiler species, heat stress can reduce feed consumption, growth rate and the increased mortality (Lara and Rostagno, 2013; Akbarian *et al.*, 2016). Since birds consume approximately twice as much water than feed under hot climate condition, one of the effective ways to reduce the negative impact of heat stress is to ensure the uptake of additional nutrients such as vitamins A and E (Kucuk *et al.*, 2003; Singh *et al.*, 2006; Qureshi *et al.*, 2018; Abd El-Hack *et al.*, 2019). However, vitamins A and E are inherently immiscible with water. Hence, it is important to formulate these vitamins into water-dispersible feed additives. Nuvivit K contains high-quality, water-dispersible vitamins A and E specially formulated for drinking water applications. These vitamin feed additives are produced using granulation technology, where the vitamins

are microencapsulated in a protein-sugar matrix, and then coagulated to form water-dispersible granules. Nuvivit K also contains other water soluble vitamins, and the premix forms a continuous dispersion in water that is stable over time.

Table 2. Weight of broiler chicken in the experiment

Day	Control	Nuvivit 150	Nuvivit 100
1	48.051±4.78	48.72±3.85	47.76±4.57
7	114.75±9.84	111.33±11.60	112.19±10.57
14	311.80^{a}±37.41	305.84^{ab}±36.40	296.98^{b}±37.10
21	841.2^{a}±103.3	783.8^{b}±86.8	804.8^{b}±81.9
28	1,426.6±181.7	1,402.7±178.6	1,387.9±156.4
35	2,311.1±292.6	2,337.7±252.6	2,309.9±238.5
42	2,906.7^{b}±340.5	2,977.8^{a}±327.0	2,998.6^{a}±270.2
49	3,488.3^{b}±357.3	3,647.9^{a}±387.5	3,688.4^{a}±316.7

Note: Means in the same row without common superscripts are different at P<0.05

The BW of bird of all group in first day was similar (P>0.05) (Table 2). After 7 days, the BW hovers at 111-114g with no significant difference (P>0.05). In this experiment, the BW of birds in general after 7 days was lower than expected. This could be due to the temperature and humidity condition in this experiment was higher (>30^{o}C and humidity >85%) thus affecting the performance at the starter stage more significantly. This condition also effected to the BW at 14 days (533g) and 21 days (1,012g). After changing the condition, the performance of chicken in all groups was similar the standard BW of the company, even higher (2296g). After 42 and 49 days, the BW of bird in the group fed with Nuvivit 100 was higher than the control (P<0.05) and there was no different in the treatment groups (P>0.05). A supplement of vitamin A (15000 IU/kg) and vitamin E (250 mg/kg) increased BW under heat stress condition (>32^{o}C) (Sahin *et al.*, 2001).

Table 3. Weight gain of broiler chicken

Week	Control	Nuvivit 150	Nuvivit 100
1	9.53^{a}±1.18	8.94^{b}±1.52	9.20^{ab}±1.42
2	28.15^{a}±4.21	27.79^{ab}±3.92	26.40^{b}±4.34
3	75.63^{a}±10.24	68.28^{b}±9.07	72.54^{a}±7.97
4	83.63±13.51	88.41±15.08	83.30±12.14
5	126.56^{b}±26.18	133.74^{a}±32.06	131.72^{ab}±20.11
6	85.08^{b}±27.29	91.44^{ab}±45.78	98.38^{a}±20.52
7	83.08^{b}±25.90	95.73^{a}±31.02	98.54^{a}±27.61

The ADG in the control group was higher than Nuvivit K at 100ppm in second week (P<0.05) (Table 3). However, in week 5 the ADG was changed and the ADG was higher in treatment group (P<0.05). In week 6 and 7, the ADG in treatments group were higher (P<0.05). This result showed that the vitamin A and E in drinking water affected the WG of chickens under stress conditions. Some other authors also concluded that dietary vitamin A and E supplementation significantly improved broiler growth performance and carcass composition and reduced heat stress related mortality (Horváth and Babinszky, 2018; Calik *et al.*, 2022).

Table 4. Feed conversion ratio of broiler chicken

Week	Control	Nuvivit 150	Nuvivit 100
1	1.11^{b}±0.03	1.12^{b}±0.05	1.24^{a}±0.16
2	1.19^{b}±0.04	1.23^{ab}±0.08	1.29^{a}±0.07
3	1.26±0.04	1.28±0.07	1.23±0.07
4	1.52^{ab}±0.07	1.46^{b}±0.13	1.55^{a}±0.11
5	1.56±0.17	1.50±0.134	1.44±0.05
6	2.46^{a}±0.47	2.43^{a}±0.25	2.13^{b}±0.10
7	2.58^{a}±0.22	2.30^{b}±0.26	2.27^{b}±0.19
Mean	1.67^{a}±0.08	1.70^{a}±0.10	1.59^{b}±0.06

The FCR in the Nuvivit K at 100ppm was higher in first and second week. However, the FCR during 7 weeks of treatment was lowest in 100ppm (1.59) compared to control and Nuvivit 150 (P<0.05). This result shows the effectiveness of Nuvivit K at 100ppm for best results under heat stress condition.

Table 5. Water intake of broiler chicken
(ml/bird/day)

Week	Control	Nuvivit 150	Nuvivit 100
1	25.26^{a}±0.00	25.85^{a}±1.15	23.45^{b}±1.05
2	76.19^{ab}±0.00	77.60^{a}±3.46	74.21^{b}±3.30
3	185.71^{b}±0.00	210.74^{a}±9.39	210.98^{a}±9.40
4	266.17±17.77	265.44±13.66	259.41±11.45
5	447.11±24.06	462.40±32.40	433.46±16.84
6	512.30±30.60	551.40±64.20	510.60±44.40
7	531.54±20.69	559.80±81.20	528.20±38.10
Mean	292.05±10.13	307.60±23.16	291.48±16.34

The water intake was different in first three week and higher in treatment groups compared to control. However, after 7 weeks, there was no difference in all groups (P>0.05),

the water intake in each group fluctuated from 291.48 to 307.15 ml/bird/day.

4. CONCLUSION

Heat stress is a major problem in tropical regions. It can be mediated by proper feeding, maintaining energy requirements and vitamins supplementation. This will improve the growth and productivity of animals through proper nutritional balance. Chicken fed with Nuvivit K at 100ppm in drinking water consistently achieved higher final weight, gain in weight and lower feed conversion ratio compared to control ($P<0.05$). Vitamins did not affect any significant differences in the water intake of the experimental birds ($P>0.05$).

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EFFECTS OF β-MANNANASE SUPPLEMENTATION WITH DIFFERENT LEVELS OF COPRA OR PALM KERNEL MEAL ON GROWTH PERFORMANCE, AND CARCASS CHARACTERISTICS IN GROWING-FINISHING PIGS

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ABSTRACT

The experiment was conducted to evaluate the effects of β-mannanase supplementation on growth performance and carcass characteristics in growing-finishing pigs fed diets containing copra or palm kernel meal. A total of 80 D(YL) pigs with an initial body weight of 20.04±0.02kg were allotted to each treatment by body weight and sex in a randomized complete block design with 4 replicates of 4 pigs/pen. The treatments were as follows: CON, a corn-soybean meal based basal diet; CM5, basal diet+5% copra meal+800IU β-mannanase; CM10, basal diet+10% copra meal+800 IU β-mannanase; PKM5, basal diet+5% palm kernel meal+800IU β-mannanase and PKM10, basal diet+10% palm kernel meal +800 IU β-mannanase. The commercial β-mannanase (CTCzyme; CTCBIO Inc., Seoul, Republic of Korea) containing 800,000IU was used in this experiment. A three-phase feeding program, consisting of a growing phase (weeks 0-6), finishing I phase (weeks 6-9), and finishing II phase (weeks 9-13), was used in this experiment. β-mannanase treatment with 5% copra meal or 5-10% palm kernel meal showed little negative effects on growth performance compared with the CON. However, in the case of the CM10 treatment, the average daily gain was significantly lower than in the other treatments ($P<0.05$). There were no significant differences in the meat pH 24hrs after slaughter. However, there was a significantly lower meat color, indicated by Hunter L* and Hunter a*, in the CM10 treatment, suggesting that 10% copra meal supplementation in swine diets may negatively impact consumer preferences for meat quality. These results indicate that 5% copra meal and 5-10% palm kernel meal can be used in growing-finishing pig diets with β-mannanase supplementation.

Keywords: *β-mannanase, copra meal, palm kernel meal, growth performance, carcass characteristics.*

1. INTRODUCTION

Recently, the global feed grain market has been steadily rising. As a result, the price of corn in the United States is now at its highest level since 1996. This increase in corn prices, along with the rising prices of soybean meal (SBM) and other feed grain ingredients worldwide, continues to push prices upward. In Korea, where up to 90% of feed grain ingredients are imported, the feed market has had to cope with a significant increase in feed costs, which has become a considerable burden for farmers. This rise in feed costs has led to an increase in pork production costs, ultimately raising consumer prices. In the United States, many researchers have investigated replacing corn and SBM with alternative feed ingredients such as DDGS (Stein and de Lange, 2007). However, in Korea, the production of alternative feed ingredients such as DDGS from bioethanol production is relatively low, so other alternative feed ingredients must be sought.

Copra and palm kernel meals are by-products, natural high-quality protein meals that are relatively inexpensive compared to corn and SBM (Kim *et al*., 2001). However, there has been a limitation in using copra and palm kernel meals for monogastric animals because they do not secrete the enzymes necessary to degrade β-mannan, making it difficult to fully digest the nutrients in the feed.

The effect of β-mannanase hydrolyzed copra and palm kernel meal has been reported to improve nutritional values

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concerning growth rate, feed efficiency, and mortality in pigs (Mok *et al*., 2013; Kwon and Kim, 2015; Kim *et al*., 2017; Jang *et al*., 2020). Consequently, this experiment was conducted to evaluate the effects of β-mannanase supplementation with different levels of copra or palm kernel meal on growth performance and carcass characteristics in growing-finishing pigs.

2. MATERIALS AND METHODS

2.1. Experimental animals and treatments

A total of 80 D(Y×L) crossbred pigs with an average body weight (BW) of 20.04±0.02kg were used for a 13wk feeding trial during the growing and finishing phases. The pigs were allotted into 5 treatment groups, with 4 replicates and 4 pigs/pen, in a randomized complete block (RCB) design. They were housed in environmentally controlled growing and finishing pens, with feed and water provided *ad libitum.*

A three-phase feeding program, consisting of a growing phase (weeks 0-6), finishing I phase (weeks 6-9), and finishing II phase (weeks 9-13), was used in this experiment. The treatments were based on the levels of copra and palm kernel meals: CON: a corn-SBM based basal diet; CM5: basal diet+5% copra meal+800IU β-mannanase; CM10: basal diet+10% copra meal+800IU β-mannanase; PKM5: basal diet+5% palm kernel meal+800IU β-mannanase; and PKM10: basal diet+10% palm kernel meal+800IU β-mannanase. Lee (2006) demonstrated that 400IU β-mannanase supplementation to growing-finishing pigs tended to show better growth performance, intestinal flora, and nutrient digestibility in corn-SBM based diets. The 800IU level of β-mannanase in the copra or palm kernel meal diets was calculated based on the higher amount of β-mannan substrate in copra or palm kernel meal compared to corn-SBM based diets. The commercial β-mannanase (CTCzyme; CTCBIO Inc., Seoul, Republic of Korea) containing 800,000IU, which was produced by *Bacillus subtilis* (WL-7) grown in Luria broth, was used at a level of 0.1% in all treatments. The chemical composition of the basal diet for all phases (growing phase, finishing I phase, finishing II phase) are presented in table 1.

Table 1. Chemical composition of the basal diet

Criteria	Growing	Finishing I	Finishing II
ME, kcal/kg	3269.72	3294.65	3323.73
CP, %	18.00	15.50	13.20
Lysine, %	0.95	0.75	0.60
Methionine, %	0.26	0.24	0.22
Ca, %	0.60	0.50	0.45
Total P, %	0.50	0.45	0.40

2.2. Measurements

Growth performance: The BW was measured at the end of each phase. In addition, the feed provided to all pigs was recorded daily, and the feed waste in the feeders was recorded at the end of each phase. Using these data, the average daily gain (ADG), average daily feed intake (FI), and gain-to-feed ratio (G:F ratio) were calculated.

Carcass characteristics: Three pigs/treatment, with an BW of 105.52±1.89kg, were selected for the evaluation of the physicochemical characteristics of pork after a 13wk feeding trial in growing-finishing pigs. The longissimus muscle area was collected at the abattoir. The collected muscle was immediately transported in a liquid nitrogen tank and kept at -60°C until analysis. Hunter L*, a* and b* values were recorded using a chromameter (Minolta, CM-5081, Japan) at 3, 6, 12 and 24h postmortem. Muscle pH was measured using a pH meter (Model 720, ThermoOrion, USA) at 24h postmortem.

2.3. Statistical analysis

All of the collected data were subjected to least squares mean comparisons and evaluated with the General Linear Model (GLM) procedure of SAS (2004). Differences among means were declared significant at $P<0.05$ and highly significant at $P<0.01$, and the determination of tendency for all analyses was $P\geq0.05$ and $P<0.10$.

3. RESULTS AND DISCUSSION

3.1. Growth performance

The effect of β-mannanase supplementation on growth performance of growing-finishing pigs fed copra or palm kernel meal is shown in table 2.

In the growing phase and finishing phase I, there were no significant differences in BW, ADG, FI and G:F ratio among all treatments. During finishing phase II, the PKM10 treatment showed significantly higher BW and ADG than the CON, CM5 and CM10 treatments. The CM10 treatment showed a significantly lower ADG than the PKM5 and PKM10 treatments. However, there were no significant differences in ADFI and G:F ratio among all treatments in finishing phase II. Throughout the entire finishing phase, significantly improved ADG was observed in the PKM5 treatment compared to the CON and CM10 treatments. Finally, in the whole experimental phase, the PKM5 treatment showed a higher BW compared with the CM10 treatment, and there were no significant differences in ADG, FI and G:F ratio among the treatments.

Table 2. Effects of β-mannanase supplementation on growth performance

Criteria		Treatment[1]					SEM[2]	p-value[3]		
		CONT	CM5	CM10	PKM5	PKM10		CM vs PKM	Adding level	Interaction
BW, kg	Initial	---------- 20.04 ----------								
	6	57.92	57.75	55.64	57.51	57.93	0.96	0.43	0.52	0.26
	9	80.13	82.29	76.83	80.87	79.04	1.10	0.85	0.04	0.23
	13	104.26^{ab}	105.47^{a}	100.28^{b}	108.37^{a}	105.04^{ab}	1.27	0.07	0.04	0.56
ADG, g	0-6	899	898	848	888	902	14.33	0.44	0.53	0.24
	6-9	1,058	1,169	1,009	1,112	1,006	27.41	0.57	0.03	0.62
	9-13	862^{bc}	828^{c}	837^{bc}	982^{a}	928^{ab}	22.36	0.01	0.51	0.34
	6-13	946^{b}	974^{ab}	911^{b}	1038^{a}	961^{ab}	16.96	0.01	0.04	0.72
	0-13	924	939	882	968	934	11.92	0.01	0.04	0.61
FI, g	0-6	2,042	2,022	1,916	1,908	1,945	38.21	0.45	0.62	0.23
	6-9	2,826	3,118	2,886	3,091	2,957	49.87	0.82	0.08	0.62
	9-13	3,335	3,201	3,354	3,505	3,314	55.30	0.30	0.92	0.18
	6-13	3,117	3,165	3,154	3,329	3,161	38.88	0.32	0.29	0.36
	0-13	2,620	2,638	2,582	2,673	2,600	28.62	0.64	0.22	0.91
G:F ratio	0-6	0.442	0.444	0.444	0.467	0.464	0.005	0.04	0.83	0.85
	6-9	0.378	0.375	0.351	0.360	0.340	0.007	0.31	0.11	0.85
	9-13	0.260	0.258	0.250	0.280	0.281	0.006	0.03	0.72	0.72
	6-13	0.305	0.307	0.289	0.312	0.305	0.004	0.01	0.08	0.53
	0-13	0.354	0.356	0.342	0.363	0.360	0.006	0.01	0.15	0.37

Note: Means with different superscripts in the same row significantly differ (P<0.05).

Additionally, the palm kernel meal treatments significantly improved ADG and G:F ratio compared to the copra meal treatments during finishing phase II, the entire finishing phase, and the whole experimental phase.

The most abundant anti-nutritional factor in copra and palm kernel meal is β-mannan (pure mannan, galactomannan, glucomannan, etc.) (Veum and Odle, 2000). The exogenous enzyme β-mannanase can degrade β-mannan into MOS or mannose, which can then serve as an energy source and prebiotics in the gastrointestinal tract (Halas and Nochta, 2012). Therefore, β-mannanase supplementation may improve the availability of copra or palm kernel meal in growing-finishing pigs. Particularly, there is more β-mannan in palm kernel meal than in copra meal (Kwon and Kim, 2015; Kiarie *et al.*, 2021), so the effect of dietary β-mannanase on growth performance is greater with palm kernel meal than with copra meal.

The lowest growth performance observed in the CM10 treatment could also be attributed to the relatively low content of β-mannan. Additionally, copra meal is known to be susceptible to a high incidence of mold growth, such as *Aspergillus spp.*, and aflatoxin contamination, which can exceed normally permitted levels when pigs are fed copra meal (Thorne *et al.*, 1990).

These results indicate that the use of 800IU of β-mannanase supplementation in diets containing 5% copra meal or 5-10% palm kernel meal based on corn-SBM did not have any negative impacts on the growth performance of growing-finishing pigs.

3.2. Carcass characteristics

The effect of β-mannanase supplementation on meat pH measured 24hrs post-slaughter of growing-finishing pigs fed copra or palm kernel meal is shown in figure 1.

There were no significant differences in pH levels when comparing the control group with the copra and palm kernel meal groups at various levels. The pH change in pork is a very significant factor that determines pork quality and influences freshness, water holding capacity, tenderness, binding ability, meat color, texture, and storage life (Palansky and Nosal, 1991; Perry, 2009). In practice, the initial pH is regarded as an indication of PSE meat, while the final pH is recognized as an estimate of DFD meat (Van *et al.*, 1988). The effect of β-mannanase supplementation on meat color, especially Hunter values, of growing-finishing pigs fed copra or palm kernel meal is shown in table 3.

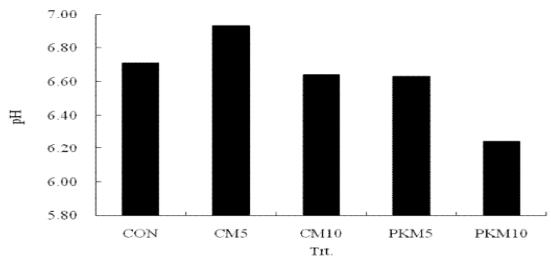

Figure 1. Effects of β-mannanase on meat pH

Table 3. Effects of β-mannanase supplementation on meat color

Criteria	Times	Treatment					SEM	P		
		CONT	CM5	CM10	PKM5	PKM10		CM vs PKM	Adding level	Interaction
Hunter L*	3h	50.33^{a}	46.96^{ab}	42.17^{b}	49.76^{a}	49.98^{a}	1.013	0.03	0.33	0.28
	6h	50.32^{a}	44.98^{ab}	42.69^{b}	49.66^{ab}	49.39^{ab}	1.074	0.04	0.63	0.71
	9h	49.58^{ab}	45.26^{bc}	43.35^{c}	52.43^{a}	50.30^{ab}	0.982	0.01	0.33	0.96
	12h	49.85^{ab}	49.39^{ab}	46.63^{b}	53.32^{a}	51.81^{ab}	0.863	0.01	0.22	0.71
	24h	47.83^{bc}	46.42^{c}	46.39^{c}	54.21^{a}	52.17^{ab}	0.800	0.01	0.39	0.40
Hunter a*	3h	3.43^{a}	3.99^{a}	1.61^{b}	2.93^{a}	3.12^{a}	0.220	0.65	0.03	0.01
	6h	4.79^{a}	5.42^{a}	2.55^{b}	3.81^{ab}	4.84^{a}	0.280	0.60	0.16	0.01
	9h	5.13^{a}	4.89^{a}	2.88^{b}	4.21^{ab}	4.59^{ab}	0.268	0.41	0.19	0.06
	12h	5.88^{a}	4.89^{ab}	2.35^{c}	4.74^{ab}	4.24^{b}	0.279	0.13	0.01	0.07
	24h	5.45^{a}	5.58^{a}	2.83^{b}	5.11^{a}	5.19^{a}	0.275	0.12	0.03	0.02
Hunter b*	3h	6.64^{a}	6.31^{a}	4.91^{b}	6.78^{a}	6.62^{a}	0.204	0.03	0.12	0.21
	6h	7.36^{a}	6.75^{ab}	5.14^{b}	6.74^{ab}	7.49^{a}	0.281	0.09	0.52	0.09
	9h	7.58^{a}	6.55^{ab}	5.85^{b}	7.76^{a}	7.85^{a}	0.266	0.01	0.57	0.47
	12h	8.41^{a}	7.30^{ab}	5.93^{b}	8.48^{a}	7.75^{a}	0.245	0.01	0.02	0.46
	24h	7.39^{ab}	7.18^{ab}	6.04^{b}	8.58^{a}	8.24^{a}	0.239	0.01	0.08	0.34

CM10 treatment showed significantly lowest Hunter L, a, b values across all phases ($P<0.05$). When consumers select pork at a market, meat color is considered the primary factor in their decision. In particular, Hunter a value is very sensitive to consumers when they assess meat quality. Therefore, even with β-mannanase supplementation, the inclusion of copra meal up to 10% can negatively affect the Hunter a value, potentially leading to a negative visual impact on consumer's choice.

Consequently, these carcass characteristic results indicate that diets

containing 10% copra meal had some negative impacts on consumer choice, even with 800 IU β-mannanase. Therefore, 5% copra meal or 5-10% palm kernel meal with 800 IU β-mannanase did not show any negative effects on carcass characteristics of growing-finishing pigs.

4. CONCLUSION

A diet with 5% copra meal and 5-10% palm kernel meal, supplemented with 800IU of β-mannanase, had no negative impact on the growth performance and carcass characteristics of growing-finishing pigs. These results indicate that 5% copra meal and 5-10% palm kernel meal can be used as a feed alternatives in growing-finishing pig diets with 800IU β-mannanase supplementation.

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EFFECT OF POWDERED HERBAL MIXTURE SUPPLEMENTS IN THE DIET ON GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL PARAMETERS OF TIEN YEN CHICKEN

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ABSTRACT

This study was conducted on Tien Yen castrated male and female chickens from Jan 2024 to Jul 2024 in Tien Yen, Quang Ninh. The Tien Yen chickens at age of 13ws old were randomly divided into 4 groups, each group containing 50 castrated male and 50 female chickens. The chickens were fed a basal diet with different levels of herbal mixture supplementation (HMS) respectively: 0% CG, 1% TG1, 1.5% TG2 and 2% TG3. Chickens fed the diets with HMS had the higher growth performance compared to those fed the diets without HMS (P<0.05). The chickens in TG3 had the higher growth performance compared to those of TG1 and TG2 (P<0.05). The growth performance of castrated male and female chickens were higher than chickens of CG by 10.41 and 9.58% respectively. Supplementing 2% herbal mixture in the diet had a better effect on improving some blood parameters of Tien Yen chickens such as the reduction of total cholesterol and liver enzymes compared to the 1.0 and 1.5% supplementation levels. The castrated male and female chickens fed the diets with 2% HMS had the total cholesterol lower 51.97-63.97% respectively compared to chickens fed the diet without HMS. The liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in the blood of castrated male chickens were decreased by 53.36-29.83%, respectively and for female chicken were decreased by 54.99-36.53%, respectively compared to those of chickens without HMS.

Keywords: *Herbal mixture, Bidens pilosa L., Cinnamon, Anise. growth performance.*

1. INTRODUCTION[1]

In recent years, researchers have particularly interested in supplementing herbal plants to feed in order to improve animal health, productive performance and livestock production efficiency. Vu Dinh Ton *et al.* (2024) studied the supplementation of herbal mixture containing *Bidens pilosa L.*, anise and cinnamon in the diet for crossbred broiler male chickens F1(DT×LP) showed that growth performance and FCR were improved, the blood cholesterol and liver function of chickens were decreased compared to those of control group. According to Yang *et al.* (2015), supplementing *Bidens pilosa L.* in the diet for chickens at different levels: 0.5, 1 or 5% had reduced the incidence of coccidiosis and improving the growth performance of chickens. According to Al-Kassie (2009), chickens' growth performance had been improved when they are fed the diet with 1.0% anise powder supplements. According to Soltan *et al.* (2008), the physiological and biochemical parameters of blood and liver function of chickens were improved when they were fed the diets supplemented with 0.5g anise/kg of feed. According to Vu Quynh Huong *et al.* (2023), adding cinnamon powder at 2.0-2.5 g/kg of feed for crossbred male chickens F1(DT×LP) improved the growth performance, some physiological and biochemical parameters of blood (cholesterol, liver enzymes, white blood cells). Almost of studies on herbal supplementation in chicken feed rations mainly use the form of extracts, essential oils and individual herbal supplements. This study aims to evaluate the effects of powered herbal mixtures including *Bidens pilosa L.*, anise and cinnamon supplemented in the diet on chickens' growth and some blood biochemical indexes.

2. MATERIALS AND METHODS

2.1. Materials and methods

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The experiment was carried on Tien Yen chickens from Jan 2024 to Jul 2024 in Tien Yen, Quang Ninh province. The Tien Yen chickens at age of 13ws old were randomly divided into 4 groups, 50 castrated males and 50 females per group (Table 1).

Table 1. Experimental Design

Group	Control group (CG)	TG1	TG2	TG3
Basal diet	Basal diet	Basal diet	Basal diet	Basal diet
Ratio of herbal mixture supplement	0	1%	1.5%	2%
Castrated male chickens	50 heads	50 heads	50 heads	50 heads
	Experiment from 13-26 weeks of age			
Female chickens	50 heads	50 heads	50 heads	50 heads
	Experiment from 13-20 weeks of age			

Table 2. Ingredients of the basal diets

Ingredients	%	Ingredients	%
Yellow corn	62	Limestone powder	2
Soybean meal	25	Dicalcium phosphate	1
Wheat bran	3	Mineral premix	1
Rice bran	4.2	DL-Methionine 98 %	0.5
		Salt powder (NaCl)	0.3
		L-Lysine HCl 70%	0.5
		L-Threonine 98%	

Experimental chickens were fed a basal diet (Table 2), the herbal mixture consisted of *bindens Pilosa L.* 80%, anise 5% and cinnamon 15%. All herbs were ground into powder and mixed into a herbal mixture, then supplemented into the chicken diet.

The nutritional value and composition of the experimental batches were analyzed at the Institute of Environmental Science and Technology.

Table 3. Nutritional value and chemical composition of the experimental diets (Mean±SE, n=3)

Calculated composition	CG	TG1	TG2	TG3
CP (%)	16.13±0.11	16.07±0.68	16.43±0.16	16.35±0.20
Lipit (%)	4.79±0.39	4.41±0.22	4.73±0.94	4.77±0.09
CF (%)	3.66^{b}±0.23	4.31^{a}±0.55	4.37^{a}±0.83	4.43^{a}±0.58
Starch (%)	47.60±0.61	47.70±0.32	47.50±0.52	46.96±0.31
Glucose (%)	5.33±0.14	5.34±0.08	5.31±0.12	5.27±0.05
ME (kcal/kg VCK)	3.055.93±22.45	3.026.57±10.75	3.056.87±11.84	3.051.32±9.80

Three samples taken out from each experimental lot were analyzed the chemical composition and nutritional value. The CP, lipid, CF, starch, sugar, ME were analyzed according to the instructions of Vietnamese National Standards (TCVN) 4328:2007, TCVN 4331:2001, TCVN 4329:2007, TCVN 9587:2013, TCVN 10327:2014, TCVN 8762:2012.

The male chickens were castrated at 45 days of age. Tien Yen chickens at age of 1-12 weeks were fed with complete industrial feed. During the experimental period, castrated male and female chickens were raised together, the flock was raised in the same chicken house, the floor was covered with rice husks. Chickens were raised in a semi-free-range method, with a density of 5 chickens/m^2 of housing's floor and 1 chicken/2m^2 of playground. Chickens were fed *ad libitum* and access to water freely.

2.2. Effect of herbal on growth performance

Each batch of 30 castrated male and 30 female chickens were numbered, which were weighed weekly by a scale (accuracy±20g). Survival rate, chicken's weight were recorded weekly. Average daily gain (ADG) was calculated basing on weekly weight gain.

2.3. Biochemical analysis of chicken blood

The six blood samples of castrated male chickens at 26w of age and 6 blood samples of female chickens at 20w of age were collected from each batch to evaluate some biochemical blood parameters. Chicken blood samples were collected in the morning,

before feeding. Each sample was 2ml of blood, then put in a test tube containing anticoagulant solution (EDTA-Ethylene-Diamine Tetra-Acetic acid) stored at 4°C and analyzed within 24h. Protein, Total cholesterol, AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase) were analyzed by the Technicon RA 1000 automatic analyzer (Technicon Instruments Corporation, Tarrytown, New York, USA).

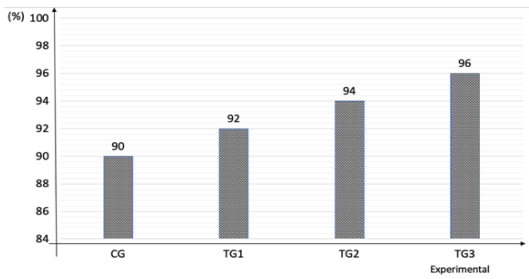
2.4. Statistical analysis

The results of this study were analyzed for differences in the mean values of the following parameters: feed chemical compositions, BW (g/head), ADG (g/day) and blood biochemistry between batches using ANOVA on SAS 9.1 software and testing the mean differences between batches using Waller-Ducan K-ratio t-Test with a significance level of P<0.05. Statistical parameters include mean values (Mean) and standard errors (SE).

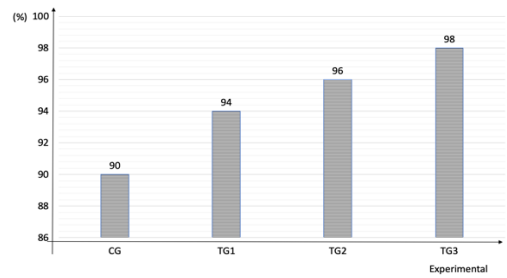
3. RESULTS AND DISCUSSION

The survival rate (SR) of Tien Yen castrated male and female chickens of experimental diets is presented in figure 1.

The research results showed that herbal mixture supplemented to the diet improved the SR of Tien Yen castrated male chickens by 2-6% and that of female ones by 4-8% compared to the control group without herbal mixture supplements. The SR was the highest in the TG3. According to Yang *et al*. (2015), adding *Bindens pilosa* L. compounds to the diet of growing chickens at levels of 0.5, 1 and 5% after 21 days has the effect of improving the SR when chickens are infected with coccidiosis. The weight of Tien Yen castrated male and female chickens at different week of age in the period of experiment is presented in tables 4 and 5.



Survival rate of castrated male chickens 14-26w



Survival rate of female chickens at 14-20w

Figure 1. Survival rate of Tien Yen chickens during the experimental period

Table 4. Tien Yen castrated male weight (n=30)

Age, w	**CG**	**TG1**	**TG2**	**TG3**	**P**
13	2,133.33 ±17.50	2,106.67±23.94	2,180.00±23.68	2,140.00±22.83	0.134
14	2,230.67±17.25	2,217.33±22.84	2,296.00±22.57	2,260.00±23.29	0.055
15	2,353.33^{b}±21.29	2,360.00^{b}±30.92	2,446.67^{a}±20.19	2,414.67^{a}±28.03	0.030
16	2,500.00^{b}±21.44	2,526.67^{b}±24.88	2,620.00^{a}±16.88	2,593.33^{a}±27.51	<0.001
17	2,660.00^{b}±13.21	2,704.00^{b}±29.53	2,804.00^{a}±15.17	2,785.33^{a}±19.53	<0.001
18	2,813.33^{b}±13.33	2,866.67^{b}±25.96	2,973.33^{a}±12.62	2,964.00^{a}±26.95	<0.001
19	2,926.67^{c}±14.33	2,993.33^{b}±25.79	3,106.67^{a}±8.21	3,113.33^{a}±22.35	<0.001
20	2,978.67^{c}±12.46	3,054.67^{b}±24.01	3,176.00^{a}±7.57	3,192.00^{a}±19.50	<0.001
21	3,013.33^{c}±15.91	3,097.33^{b}±22.80	3,226.67^{a}±7.75	3,253.33^{a}±18.22	<0.001
22	3,038.67^{c}±5.06	3,126.67^{b}±23.08	3,260.00^{a}±6.91	3,301.33^{a}±19.72	<0.001
23	3,054.67^{d}±15.00	3,149.33^{c}±24.25	3,288.00^{b}±7.15	3,334.67^{a}±19.05	<0.001
24	3,069.33^{d}±15.69	3,166.67^{c}±23.55	3,310.67^{b}±7.41	3,365.33^{a}±17.23	<0.001
25	3,080.00^{d}±14.66	3,180.00^{c}±24.33	3,328.00^{b}±7.64	3,389.33^{a}±17.46	<0.001
26	3,086.67^{d}±14.45	3,189.33^{c}±23.28	3,341.33^{b}±7.48	3,408.0^{a}±17.77	<0.001

Note: Means with different superscripts in the same row are significantly different at P<0.05.

After two weeks of experiment, the BW of the castrated male chicken in the TG2 and the TG3 was higher than that of CG and TG1 (P<0.05) (Table 4). After six weeks of experiment (19 weeks of age), the BW of chickens fed diets with HMS was higher than that of CG (P<0.05). At the end of the experience the BW of Tien Yen castrated male chickens in TG3 was 2.0% higher than that of TG2, 6.86% higher than that of TG1 and 10.41% higher than that of CG.

Table 5. Tien Yen female weight by age (n=30)

Age,w	**CG**	**TG1**	**TG2**	**TG3**	**P**
13	1,600.00 ±13.56	1,613.33 ±11.48	1,606.67 ±14.33	1,600.00 ±16.60	0.893
14	1,692.00 ±11.96	1,717.33 ±11.65	1,718.67 ±15.54	1,717.33 ±17.09	0.485
15	1,800.00 ±12.05	1,833.33 ±18.77	1,846.67 ±16.42	1,853.33 ±21.29	0.143
16	1,902.67^{b} ±14.47	1,946.67^{ab} ±20.19	1,970.67^{a} ±17.93	1,986.67^{a} ±23.35	0.015
17	1,965.33^{c} ±15.78	2,022.67^{b} ±22.60	2,053.33^{ab} ±19.01	2,080.00^{a} ±21.65	0.001
18	2,010.67^{c} ±17.20	2,080.00^{b} ±30.71	2,118.67^{ab} ±22.34	2,153.33^{a} ±22.47	0.001
19	2,034.67^{c} ±18.26	2,117.33^{b} ±34.25	2,162.67^{ab} ±20.42	2,208.00^{a} ±21.04	<0.001
20	2,046.67^{c} ±17.29	2,136.00^{b} ±34.31	2,188.00^{ab} ±18.83	2,242.67^{a} ±17.88	<0.001

After 3 weeks of experiment (16w of age), the BW of female chickens in TG2 and TG3 was higher than that of CG (P<0.05). Meanwhile, the BW of female chickens in TG1 was higher than that of CG after 4w of experience (P<0.05). At the end of the experience, the BW of chickens in TG3 was higher than that in TG1 and CG by 4.99 and 9.58%, respectively; the BW of the chickens in TG2 and TG1 was 6.91 and 4.36% higher than that in CG, respectively.

The results of this study showed that herbal mixture supplemented to the diet improved the growth of Tien Yen castrated male and female chickens compared to the chickens fed the diets without herbal mixture supplements. The 2% herbal supplement level was the best level to improve the growth of Tien Yen castrated male chickens. While, 1.5 and 2% herbal mixture supplements had a good effect on improving the growth of female chickens. The results of our study are similar to the results of some researchers when adding herbs to the chicken diet. According to Al-Kassie (2009), cinnamon contains active ingredients (cinnamaldehyde and ugenol) which have the positive effect on the growth of chickens. According to Memon *et al.* (2021), using a *Bindens pilosa* L. supplement at a dose of 0.5%/kg of feed improved the growth performance of chickens. According to Eltazi (2014), adding 1% anise powder to the diet improved the growth performance of chickens. According to Lee *et al.* (2004), who found that supplementing cinnamon to the diet of broiler chickens improved their growth performance. The ADG of Tien Yen castrated male and female chickens during the experimental period is shown in figures 2 and 3.

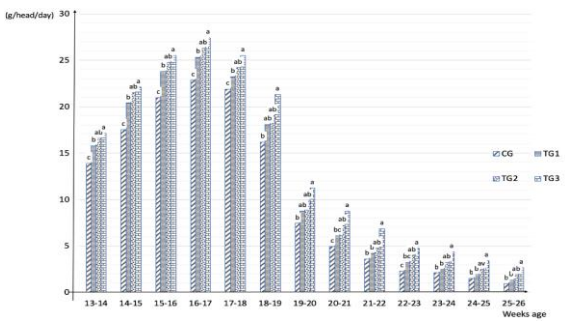


Figure 2. ADG of castrated male chickens (13-26w age)

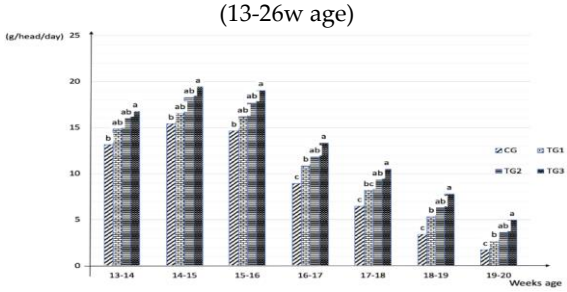


Figure 3. ADG of female chickens (13-20w age)

Figures 2 and 3 show that the ADG of the chickens fed diets with herbal mixture supplements was higher than that of the CG without HMS (P<0.05). Herbal supplementation at 1.5 and 2% had a better

effect on the growth rate of Tien Yen castrated male and female chickens than the 1% herbal supplementation level (P<0.05). According to Bedford (2000), herbal products can control the growth of bacteria in the small intestine of chickens, thereby contributing to improving the growth performance of chickens. According to Vidanagamage *et al.* (2016), adding cinnamon powder to the diet reduces stress and improves the growth performance of chickens. According to Vu Dinh Ton *et al*. (2024), adding a mixture of herbs including *Bindens pilosa L*., anise and cinnamon had the positive effect on the growth performance of crossbred male chickens F_1(DT×LP) by 7.7-16.9%. Some biochemical indexes of chicken blood are presented in tables 6 and 7.

Table 6. Biochemical parameters of castrated male 26w

Criteria	CG	TG1	TG2	TG3	P
Protein, mg/100ml	4.04 ±0.16	4.03 ±0.01	4.05 ±0.01	4.05 ±0.02	0.83
Total Cholesterol, mg/100ml	199.71^{a} ±6.20	143.65^{b} ±11.66	90.36^{c} ±3.15	71.96^{c} ±3.64	<0.001
Aspartate Aminotransferase, U/l	361.50^{a} ±10.57	270.98^{b} ±4.94	255.89^{b} ±9.14	168.60^{c} ±6.32	<0.001
Alanine Aminotransferase, U/l	28.00^{a} ±0.42	23.58^{b} ±0.75	20.83^{bc} ±0.80	19.65^{c} ±0.68	<0.001

Table 7. Biochemical parameters of female' blood at 20w

Criteria	CG	TG1	TG2	TG3	P
Protein, mg/100ml	4.03 ±0.02	4.02 ±0.01	4.02 ±0.01	4.03 ±0.02	0.051
Total Cholesterol, mg/100ml	132.63^{a} ±5.56	127.15^{a} ±11.73	86.15^{b} ±5.78	63.70^{c} ±3.08	<0.001
Aspartate Aminotransferase, U/l	370.16^{a} ±14.61	302.17^{b} ±21.84	239.83^{c} ±10.23	166.62^{d} ±15.92	<0.001
Alanine Aminotransferase, U/l	30.33^{a} ±2.01	25.41^{b} ±1.93	24.00^{b} ±0.57	19.25^{c} ±1.92	<0.001

Tables 6 and 7 show that adding herbs to feed have the effect of reducing total cholesterol and liver enzymes in chicken blood. For castrated male chickens, the amount of cholesterol in blood of the TG3 was decreased by 63.97% compared to that of chickens of CG without herbal mixture supplement, decreased by 49.91% compared to TG1 and decreased by 20.36% compared to TG2. There was significant difference of this parameter between the TG3, TG2 and TG1 and CG (P<0.05). For the female chickens, the amount of cholesterol in chicken blood belonging to TG3 was decreased by 51.97, 49.9 and 26.6% compared to that of the CG, TG1 and TG2. There was significant difference of this parameter between the TG3 and TG2, between TG3, TG2 and TG1, CG (P<0.05). There was not significant difference of this parameter between TG1 and CG (P>0.05). The results of our study are similar to the results of Paryad and Mahmoudi (2008), which showed that adding cinnamon essential oil to the diet reduced total cholesterol and LDL cholesterol in chicken blood.

The amount of liver enzymes AST and ALT in the blood of castrated male chickens in TG3 were decreased by 53.36 and 29.83% compared to those of CG; decreased by 37.78 and 16.67% compared to those of TG1 and decreased by 34.11 and 5.66% compared to TG2. There was significant difference on the AST parameter between experimental groups (P<0.05) but there was only significant difference on ALT parameter between TG3 and TG1, CG (P<0.05); between TG2, TG1 and CG (P<0.05). There was not significant difference between TG3 and TG2 (P>0.05); and between TG1 and TG2 (P>0.05).

The AST and ALT in the blood of female chickens in TG3 were decreased by 54.99 and 36.53% respectively compared to those of CG; decreased by 44.86 and 24.24% compared to those of TG1 and decreased by 30.53 and 19.79% compared to TG2. There was significant difference on the AST parameter between experimental groups (P<0.05) but there was only significant difference on ALT parameter between TG3 and TG2, TG1, CG (P<0.05), it was not significant difference between TG1 and TG2 (P>0.05). According to Langhout (2000), when herbs supplemented to the diets, liver function can be improved.

4. CONCLUSIONS

Herbal mixture in powder form including *Bidens pilosa L.*, Anise and Cinnamon supplemented to the diet has the good effect on improving the growth of Tien Yen castrated male and female chickens. The 2% HMS level had a better effect on growth, it has improved the growth of castrated male and female chickens by 10.41 and 9.58%, respectively.

Supplementing 2% herbal mixture in the diet had a better made reducing total cholesterol and liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in the blood of female and castrated male chickens.

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EFFECTS OF SUPPLEMENTATION OF POWDERED HERBAL MIXTURE AND CINNAMON IN THE DIET ON PRODUCTIVITY AND QUALITY OF TIEN YEN CHICKEN MEAT

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ABSTRACT

This study investigates the effects of supplementation with a powdered herbal mixture, and cinnamon, on the productivity and quality of Tien Yen chicken meat. The research involved selecting castrated male chickens at 26 weeks and female chickens at 20 weeks, ensuring that the chosen chickens were representative of their flock's average weight. Key indicators of meat yield such as live weight, carcass weight, breast meat weight, and thigh meat weight were meticulously evaluated, alongside measurements of carcass, breast, thigh, and belly fat percentages. Additionally, the quality of the meat was assessed based on pH levels, color, toughness, water loss, and the content of lipids and cholesterol. The findings revealed that supplementing the chickens' diet with herbs and cinnamon powder led to significant improvements in productivity and meat quality, particularly through reduced weight and belly fat percentages and lower lipid and cholesterol levels in the breast meat. The most effective results were observed with a 2% herbal supplement, followed by 1.5% herbal and 0.2% cinnamon supplements. These results underscore the potential benefits of incorporating herbal and cinnamon additives into poultry diets as a strategy for enhancing meat yield, quality, and healthiness.

Key words: *Herbal, meat quality, Tien Yen chickens, productivity, cholesterol.*

1. INTRODUCTION

In recent years, the livestock industry in our country has experienced rapid and robust growth, particularly in chicken farming. However, this expansion has brought challenges for chicken farmers, including rising feed costs and increased risks of poultry diseases, which, in turn, elevate veterinary expenses. Major diseases such as African swine fever and avian influenza have added to these concerns. To improve livestock survival rates and prevent diseases, farmers often resort to mixing antibiotics into animal feed and drinking water.

A study by Coyne *et al.* (2019) on antibiotic use in livestock farming in Thailand, Indonesia, and Vietnam found that 54% of farms used antibiotics to reduce mortality and promote chicken health, 33% to enhance chicken growth, and 20% to prevent diseases. However, using antibiotics for disease prevention or in incorrect dosages, particularly without observing withdrawal periods before slaughter, increases the risk of antibiotic resistance and antibiotic residues in chicken meat. Nguyen Thi Lan *et al.* (2016) investigated the antibiotic resistance of Ornithobacterium rhinotracheale (ORT), a bacterium causing respiratory issues in chickens. The study revealed that ORT was resistant to 8 out of 14 antibiotics (57.14%), with 100% of samples resistant to erythromycin, gentamycin, enrofloxacin, and norfloxacin, and high resistance rates to other antibiotics such as manumycin (93.3%), tylosin (83.33%), colistin (96.67%) and lincomycin (70%).

To address these issues, one effective solution in animal production is the use of herbal supplements in animal feed. Incorporating herbs into feed not only reduces the reliance on synthetic antibiotics but also enhances animal husbandry efficiency by improving growth performance, increasing feed efficiency, and enhancing the quality of livestock products. This approach has gained traction globally, including in

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Vietnam. Herbs, known for their medicinal and health-boosting properties, can improve livestock health. According to Mirzaei-Aghsaghali (2012), herbs exhibit antibacterial activity, boost immunity, improve feed efficiency, and reduce disease incidence in livestock.

Vietnam, with its rich diversity of herbal plants, is well-positioned to benefit from this trend. Popular herbs such as star anise, cinnamon, needlewood, and pearl grass are widely cultivated or grow wild (needlewood) in many regions. Several global studies (Memon *et al.*, 2020; Eltazi, 2014; Al-Kassie, 2009; Alikwe *et al.*, 2014; Shahrajabian *et al.*, 2020) have explored the use of these herbs as supplements in animal feed and drinking water, further validating their potential benefits.

2. MATERIALS AND METHODS

2.1. Materials and methods

This study was conducted on Tien Yen capons and hens from Jan 2024 to Jul 2024 in Tien Yen district, Quang Ninh province, Vietnam. 13-week-old Tien Yen chickens, including 250 capons and 250 female chickens, healthy and uniform in weight, were randomly divided into 5 groups, each group containing 50 capons and 50 female chickens (Table 1).

The experimental chicken flock was fed a basal diet (BD) (Table 2) with varying levels of herbal supplementation

Table 1. Experimental designe

Lot	DC	CT1	CT2	CT3	CT4
Basal diet (BD)	BD	BD	BD	BD	BD
Herbal mixture supplements	0%	1%	1.5%	2%	0%
Cinnamon powder	0%	0%	0%	0%	0.2%
Capon (13-26ws old)	50	50	50	50	50
Female chickens (13-20w old)	50	50	50	50	50

. The control group (DC) received no herbal supplements, while the CT1 group was supplemented with 1% herbs, the CT2 group with 1.5% herbs, and the CT3 group with 2% herbs and CT4 group with 0.2% cinamon. The herbal mixture consisted of *Bidens pilosa* (80%), star anise (5%), and cinnamon (15%). These herbs were ground into a powder, blended into the herbal mixture, and then incorporated into the chicken diets.

Table 2. Ingredients of the basal diets

Ingredient	%	Ingredient	%
Yellow corn	62	Stone powder	2
Dried soybeans	25	DCP	1
Barley bran	3	Mineral premix	1
Plain rice bran	4.2	DL-Methionine 98%	0.5
		NaCl	0.3
		L-Lysine HCI	0.5
		L-Threonine	0.5

The food nutritional value and composition of the experimental batches were analyzed at the Center for Environment and Quality Testing, Institute of Environmental Science and Technology.

Table 3. Composition and nutritional value of experimental batches (Mean±SE, n=3)

Criteria	DC	CT1	CT2	CT3	CT4
CP (%)	16.1±0.1	16.1±0.7	16.4±0.2	16.4±0.2	16.1±0.7
Lipids (%)	4.8±0.4	4.4±0.2	4.7±0.9	4.8±0.1	4.6±0.1
CF (%)	3.7^{b}±0.2	4.3^{a}±0.6	4.4^{a}±0.8	4.4^{a}±0.6	3.7^{b}±0.1
Starch (%)	47.6±0.6	47.7±0.3	47.5±0.5	47.0±0.3	46.9±0.4
Road (%)	5.3±0.1	5.3±0.1	5.3±0.1	5.3±0.1	5.3±0.1
ME (Kcal/kg DM)	3055.9±22.5	3026.6±10.8	3056.9±11.8	3051.3±9.8	3048.7±6.9
Food cost (VND/kg)	9893	10643	11018	11393	9973

Each batch includes three feed samples, with each sample weighing 200 g, for analysis of the feed's composition and nutritional value. CP was analyzed according to the instructions of TCVN 4328: 2007; Lipids were analyzed according to the TCVN 4331:2001; CF was analyzed according to TCVN 4329:2007; Starch was analyzed

according to the TCVN 9587:2013; Sugar was analyzed according to TCVN 10327:2014. ME (kcal/kg DM) is calculated according to the instructions of TCVN 8762:2012.

Male chickens were castrated at 45 days of age. From 1 to 12w of age, they were fed a complete industrial mixed diet. During the experimental phase, castrated males and female chickens were raised together in an open barn with a floor covered in rice husks. The chickens were managed by semi-free-range method, with a stocking density of 5 chickens/m^2 of cage and 1 chicken/2m^2 of playground. They had free access to food, water and received uniform care.

Samples from castrated male chickens were taken at 26w of age, and from female chickens at 20w of age, to evaluate the productivity and quality of chicken meat in the experimental batches. The analysis of chicken meat yield and quality was conducted at the Center for Environment and Quality Testing, Institute of Environmental Science and Technology.

2.2. Evaluation of meat yield

At 26w of age, select 6 castrated male chickens, and at 20w of age, select 6 female chickens with an average weight representative of the overall flock in the batch. The meat yield evaluation includes the following indicators: live weight (g), carcass weight (g), carcass percentage (%), breast meat weight (g), breast meat percentage (%), weight thigh meat (g), thigh meat percentage (%), belly fat mass (g), belly fat percentage (%). These measurements were conducted according to the instructions of Bui Huu Doan *et al.* (2011).

2.3. Evaluation of meat quality

The criteria for evaluating chicken meat quality included pH, color, toughness, water-holding capacity, and post-processing loss rate. All evaluations were conducted following the guidelines of Bui Huu Doan *et al.* (2011). Meat color was assessed using a colorimeter, while the loss rate after 24hrs of storage was calculated. The pH of the meat was measured three times with a handheld pH meter (Testo 230, with an electrode), and the mean value was recorded as the sample's pH. Meat toughness was determined by measuring the shear force perpendicular to the muscle fiber axis, expressed in Newtons (N), using a Warner-Bratzler 2000D device (USA). A 100g sample of chicken breast was collected post-slaughter, weighed, vacuum-sealed in a plastic bag, and stored at 4°C. The sample was transported to the laboratory within 24hrs for analysis. Meat composition was further analyzed to determine lipid and cholesterol content.

2.4. Statistical Analysis

The results of this study were analyzed for differences in mean values between batches using ANOVA in SAS 9.1 software. The mean differences between batches were tested using the Waller-Duncan K-ratio t-Test with a significance level of $P<0.05$. Statistical parameters included mean values (Mean) and standard error (SE).

3. RESULTS AND DISCUSSION

According to Choo *et al.* (2014), chicken meat yield is influenced by breed, rearing methods, and nutritional conditions. The meat yield of Tien Yen castrated male and female chickens meat are presented in table 4 and 5.

This study found that dietary supplementation with herbs (at 1.5 and 2%) and cinnamon (at 0.2%) significantly improved the meat yield of Tien Yen chickens compared to the control group. The CT3 group, with 2% herbal supplementation, showed the highest increase in carcass weight at 11.9%, followed by CT2 (8.2%) and CT4 (5.2%) ($P<0.05$). However, no significant difference was observed between the CT1 group (1.5% herbal supplement) and the control group. Herbal supplementation did not affect the carcass percentage in castrated roosters, consistent with previous findings by Nguyen Van Duy *et al.* (2023).

Table 4. Effect of herbal mixture and cinnamon supplementation on meat yield of Tien Yen castrated male chickens at 26 weeks of age (Mean±SE, n=6)

Criteria	DC	CT1	CT2	CT3	CT4	P
Live weight, g	3060^{e}±15.9	3143.3^{d}±19.9	3313.3^{b}±17.7	3406.7^{a}±13.6	3226.7^{c}±16.3	<0.001
Carcass weight, g	2186.7^{d}±13.1	2233.3^{d}±15.7	2366.7^{b}±14.6	2446.7^{a}±14.6	2300^{c}±61.0	<0.001
Carcass ratio, %	71.5±0.5	71.1±0.3	71.4±1.0	71.8±0.7	71.3±1.2	0.4905
Thigh meat weight, g	446.6^{d}±5.4	467.7^{c}±6.6	533.7^{b}±5.1	597.4^{a}±7.8	522.7^{b}±5.7	<0.001
Thigh meat ratio, %	20.4^{c}±0.9	20.9^{c}±0.6	22.6^{b}±0.5	24.4^{a}±1.0	22.7^{b}±0.6	<0.001
Breast meat weight, g	351.2^{c}±3.8	369.6^{bc}±4.8	429.5^{ab}±6.8	475.7^{a}±6.2	404.6^{bc}±4.6	0.0100
Breast meat ratio, %	16.1^{b}±1.3	16.6^{ab}±1.5	18.2 ^{ab}±2.7	19.4^{a}±3.2	17.6^{ab}±3.2	0.1151
Belly fat weight, g	111.9^{a}±5.6	90.4^{b}±2 .6	89.7^{b}±2.2	69.7^{c}±5.4	88.5^{b}±4.3	0.0008
Belly fat, %	5.12^{a}±0.21	4.05^{b}±0.60	3.79^{b}±0.89	2.9^{c}±1.0	3.9^{b}±0.3	<0.001

Note: Means in the same row with different letters is statistically significant difference at P<0.05.

Thigh meat weight was significantly higher in all herb- and cinnamon-supplemented groups compared to the control, with the CT3 group showing the most substantial increase (33.8%) (P<0.05). Breast meat weight was also highest in the CT3 group, with increases of 35.5% compared to the control. The study suggests that 2% herbal supplementation is most effective for improving both thigh and breast meat yields, with herbs having a more pronounced effect than cinnamon. These findings align with prior research indicating that such supplementation can enhance muscle development in chickens.

Regarding belly fat, the study found that the control group had the highest belly fat mass and ratio, while the CT3 group (2% herbal supplement) had the lowest (P<0.05). There were no significant differences in belly fat among the other supplemented groups. In hens, carcass weights were higher in all supplemented groups compared to the control, with no significant differences among the varying levels of supplementation. These results support the conclusion that both herbal and cinnamon supplementation can effectively enhance meat yield and reduce belly fat in Tien Yen chickens.

Table 5. Effect of herbal mixture and cinnamon supplementation on meat yield of Tien Yen female chickens at 20 weeks of age (Mean±SE, n=6)

Criteria	DC	CT1	CT2	CT3	CT4	P
Live weight, g	2026.7^{c}±11.2	2126.7^{b}±16.3	2186.7^{ab}±11.6	2226.7^{a}±16.2	2153.3^{ab}±10.2	0.001
Carcass weight, g	1416.7^{b}±18.9	1500.0^{a}±10.0	1546.7^{a}±16.2	1566.7^{a}±17.4	1513.3^{a}±14.6	0.001
Carcass ratio, %	69.9±3.5	70.5±3.3	70.7±2.3	70.4±2.1	70.3±4.6	0.996
Thigh meat weight, g	262.0^{b}±3.5	280.3^{ab}±5.8	324.0^{a}±5.7	338.7^{a}±4.0	288.0^{ab}±3.1	0.046
Thigh meat ratio, %	18.5±0.6	18.7±0.3	21.0±1.6 0	21.6±1.7	19.03±0.5	0.171
Breast meat weight, g	206.7^{d}±11.6	216.3^{dc}±15.2	237.7^{b}±13.3	254.0^{a}±7.2	226.0^{bc}±17.1	<0.001
Breast meat ratio, %	14.6^{b}±1.0	14.4^{b}±1.7	15.4^{ab}±0.4	16.2^{a}±0.7	14.9^{b}±0.9	0.021
Belly fat weight, g	77.5^{a}±8.3	57.0^{b}±5.2	57.0^{b}±4.3	28.0^{c}±4.3	54.0^{b}±5.2	0.001
Percentage of belly fat	5.5^{a}±1.9	3.8^{b}±2.0	3.7^{b}±0.6	1.8^{c}±1.5	3.6^{b}±0.2	0.001

The study found that thigh meat weight in hens from the CT2 and CT3 supplemented groups was significantly higher than in the control group, while no significant differences were observed between the CT1, CT4, and control groups. Thigh meat percentages across all groups were consistent and higher than the range previously reported by Nguyen Van Duy *et al.* (2023). Specifically, thigh meat weight in the CT2 and CT3 groups was 23.66 and 29.26% higher, respectively, compared to the control group. Similarly, breast meat weight was higher in the CT2, CT3, and CT4 groups compared to the control group, with the CT3 group showing the most significant increase.

Additionally, the mass and percentage of belly fat were significantly lower in the

herb- and cinnamon-supplemented groups compared to the control group, with the 2% herbal supplementation being the most effective. These results indicate that dietary supplementation with herbs and cinnamon can enhance meat yield and reduce belly fat Tien Yen female chickens, with the 2% herbal supplement showing the greatest efficacy. These findings are consistent with previous research by Al-Kassie (2009) and Eltazi (2014), who reported similar improvements in meat yield and reductions in belly fat in chickens, while Shirzadegan (2014) found no significant effect of cinnamon powder on fat ratio.

The meat quality results for both Tien Yen castrated roosters and female chickens are presented in table 6 and cholesterol and lipid values are presented in table 7.

Table 6. Quality indicators of breast meat of Tien Yen castrated male and female chickens (Mean±SE, n=6)

Criteria		DC	CT1	CT2	CT3	CT4	P
Castrated male chickens at 26 weeks of age							
pH 24		5.6±0.0	5.6±0.0	5.6±0.1	5.6±0.0	5.6±0.1	0.9666
Meat color	L	57.8±0.4	57.9±0.3	58.5±0.3	58.4±0.5	57.7±0.2	0.3435
	a	12.0^{c}±0.5	12.6^{bc}±0.2	13.2^{ab}±0.4	14.1^{a}±0.4	12.8^{bc}±0.3	0.0060
	b	19.6^{c}±0.2	20.7^{b}±0.4	20.9^{b}±0.3	22.1^{a}±0.2	20.3^{bc}±0.2	0.0002
Drip loss, (%)		1.4±0.1	1.4±0.1	1.2±0.2	1.2±0.1	1.4±0.2	0.7871
Cooking loss, (%)		19.3^{a}±0.4	18.2^{b}±0.3	17.8^{b}±0.2	16.7^{c}±0.0	18.6^{ab}±0.4	<0.0001
Shear force, (N)		21.6^{c}±2.2	26.4^{b}±2.0	32.3^{a}±1.7	35.5^{a}±1.0	23.7^{bc}±1.0	<0.0001
Tien Yen female chickens at 20 weeks old							
pH 24		5.7^{b}±0.0	5.8^{b}±0.0	5.9^{a}±0.1	6.0^{a}±0.0	6.0^{a}±0.0	0.0005
Meat color	L	59.4^{c}±0.3	60.3^{bc}±0.5	60.7^{b}±0.2	62.5^{a}±0.3	61.2^{b}±0.2	<0.0001
	a	11.4^{b}±0.3	12.2^{b}±0.4	13.3^{a}±0.1	13.5^{a}±0.3	11.8^{b}±0.4	0.0008
	b	14.2^{d}±0.2	14.7^{dc}±0.3	16.9^{b}±0.2	18.5^{a}±0.2	15.4^{c}±0.2	<0.0001
Drip loss, (%)		2.2^{a}±0.1	2.0^{ab}±0.3	1.7^{ab}±0.1	1.5^{b}±0.1	1.7^{ab}±0.1	0.0088
Cooking loss, (%)		22.3^{a}±0.1	20.9^{b}±0.3	19.8^{c}±0.3	18.5^{d}±0.1	20.8^{b}±0.2	<0.0001
Shear force, (N)		21.4^{b}±0.8	22.5^{ab}±1.0	23.1^{ab}±0.4	24.1^{a}±0.3	22.7^{ab}±0.4	0.0121

Table 7. Cholesterol and lipids in breast meat of Tien Yen castrated roosters and hens (Mean±SE, n=6)

Chickens	Criteria	Unit	DC	CT1	CT2	CT3	CT4	P
Castrated male chickens at 26 weeks old	Lipids	g/100g	0.9^{a}±0.1	0.8^{b}±0.1	0.8^{b}±0.1	0.7^{d}±0.0	0.7^{c}±0.0	0.04
	Cholesterol	mg/100g	34.8^{a}±1.4	31.3^{a}±3.0	21.2^{bc}±1.9	20.3^{c}±1.6	23.9^{b}±0.7	0.02
Female chickens at 20 weeks old	Lipids	g/100g	1.0^{a}±0.1	0.9^{a}±0.1	0.8^{b}±0.3	0.6^{c}±0.1	0.8^{b}±0.1	0.04
	Cholesterol	mg/100g	26.7^{a}±0.3	23.9^{b}±0.2	23.1^{c}±0.2	18.7^{d}±0.4	22.3^{c}±0.2	0.00

This study demonstrates that herbal and cinnamon supplementation significantly reduces lipid and cholesterol levels in the breast meat of Tien Yen hens and castrated roosters compared to the control group. For castrated roosters, the CT3 group exhibited the lowest lipid content, while the CT3 and CT2 groups showed the most substantial reductions in cholesterol levels. Similarly, in hens, groups supplemented with 1.5% and 2% herbs, and 0.2% cinnamon, exhibited lower lipid and cholesterol levels compared to the control. The results highlight the potential of herbal and cinnamon supplementation to improve meat quality by reducing lipid and cholesterol levels.

The effectiveness of cinnamon, attributed to its antioxidant properties, aligns with previous findings by Shirzadegan (2014), who reported that cinnamon decreases lipid peroxidation and cholesterol levels in chicken meat. Additionally, Milićević *et al.* (2014) emphasized the importance of managing dietary cholesterol, noting its link to coronary artery disease and cardiovascular issues in humans. These findings suggest that incorporating herbs and cinnamon into the diet of Tien Yen chickens

can enhance meat quality while potentially offering health benefits to consumers.

4. CONCLUSION

Adding herbs and cinnamon powder to the diet improved meat productivity of castrated roosters and Tien Yen female chickens, reducing weight and belly fat percentage. Reduces the amount of lipids and cholesterol in the breast meat of Tien Yen castrated male and female chickens. The 2% herbal supplement level gives the best effect, followed by the 1.5% herbal supplement and 0.02% cinnamon supplement level.

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EFFECTS OF COMPOUND TRACE MINERALS ON THE GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND MEAT QUALITY OF CROSSBRED F_1(HO×LP) CHICKENS

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ABSTRACT

This study aimed to evaluate the effects of dietary supplementation with compound trace mineral (CTM) on the growth performance (GP), carcass characteristics (CC), and meat quality (MQ) of crossbred F_1(Ho×LP) chickens. This study was conducted at the experimental farm of the Faculty of Animal Science (FAS), Vietnam National University of Agriculture (VNUA) from Feb to Jul 2019. A total of 95 F_1(HxLP) chickens (54 females, 41 males) of 1 day of age were identified individually by the number on their legs and randomly allocated into one of three dietary treatments, including 0.5mg CTM (basal diet+0.5mg CTM/kg feed), 1mg CTM (basal diet+1mg CTM/kg feed), and control (basal diet). The results showed that increasing the levels of CTM in the diets did not affect the BW and ADG of chickens from birth to 5w of age. For a period of 6-18w of age, significant linear increases in the BW, and ADG were observed in chickens fed CTM diets compared with those fed the control diet. However, increasing the levels of CTM in the diets did not affect the parameters of the CC, and MQ of chickens. This suggests that the addition of 0.5mg of CTM/kg of feed is a recommended dose to improve the GP in F_1(Ho×LP) chickens.

Keywords: *F_1(HoxLP) chicken, compound trace mineral, growth performance, carcass characteristics, meat quality.*

1. INTRODUCTION

Macrominerals and microminerals (trace minerals) are important in livestock diets (National Research Council, NRC, 2012). Only a few trace minerals have been identified as essential for animal life because the lack of a few trace minerals can lead to disorders of growth and health (Acda and Chae, 2002). Minerals in the diet usually decrease with storage time leading to deficiencies in the process of using poultry's production. To prevent deficiencies in the diet of animals that need to be supplemented by a number of inorganic salts, organic metals such as iron, copper, zinc, manganese, mage and selenium... with the necessary content to maintain lead the normal development of the livestock (NRC, 2012).

Research indicates that organic and coated trace minerals can improve GP, CC, and MQ in broiler chickens compared to inorganic forms. Supplementation with organic or coated trace minerals has been shown to enhance ADG, feed conversion ratio (FCR), and antioxidant status (Yin *et al.*, 2021; Baloch *et al.*, 2017). Hydroxychloride trace minerals have demonstrated positive effects on GP and gut microbiota diversity (Van *et al.*, 2020). The use of organic zinc, manganese, and copper at reduced levels (50% of recommendations) improved GP, CC, and bone quality while decreasing mineral deposition in tissues and excreta (El-Hussein *et al.*, 2012). Additionally, the fat source in diets can influence growth and MQ, with soybean oil showing benefits over lard (Yin *et al.*, 2021). These findings suggest that organic and coated trace minerals, even at reduced levels, can effectively enhance broiler production and MQ.

The aim of the present study was to investigate the effects of dietary supplementation with CTM on the GP, CC, and MQ of crossbred F_1(Ho×LP) chickens.

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2. MATERIALS AND METHODS

2.1. Experimental design, animals and diets

The compound trace mineral (CTM) mixture used in this experiment was formulated as hydroxides from the Sea Water Chemical Institute, INC., Japan. One gram of CTM included 300mg calcium, 150mg magnesium, 25mg zinc, 15mg iron, 4mg manganese, and 2mg copper.

Table 1. Feed formulation and nutrient composition

Variables		1-4w	5-18w
Ingredient (%)	Corn	35.0	35.5
	Fish meal	12.5	10.0
	Soybean meal	17.9	11.8
	Broken rice	27.9	36.0
	Rice bran	4.8	4.8
	Vitamin premix	0.4	0.4
	Mineral premix[1]	0.5	0.5
	Calcium carbonate	0.49	0.49
	DCP	0.51	0.51
Nutrient compositions (%)	DM	86.84	89.10
	CP	21.34	18.10
	Crude fat	4.60	4.01
	CF	2.22	3.65
	Total ash	5.10	8.24
	ME (kcal/kg)	3027	3000

Note: Provided per kg of premix: $ZnSO_4$ (min-max): 1500-1800mg; $FeSO_4$ (min-max): 1500-1800mg; $MnSO_4$ (min-max): 750-800mg; $CuSO_4$ (min-max): 250-300mg; Biotin: 1.25 mg; coarse sand (max): 2%; moisture (max): 10%.

A total of 95 crossbred F_1(Ho×LP) chickens (54 females+41 males), at 1 day of age were used for this study. The chickens were raised at a experimental farm of FAS, VNUA from Mar 2019 to Jun 2019. Animals were individually notched by their legs and allocated randomly into one of three dietary treatments, including the control (basal diet), 0.5mg CTM treatment (basal diet+0.5mg CTM/kg feed), or 1mg CTM treatment (basal diet+1mg CTM/kg feed). The numbers of chickens for the control, 0.5mg and 1mg CTM/kg feed treatments were 34, 31 and 30, respectively. All experimental broilers were raised in the same housing system with the floor of the housing covered with rice husks. Feed and water were offered *ad libitum*. The density was 5 chickens/m^2 of floor area in the housing. The diet was analyzed at the Central Laboratory of the FAS, VNUA and nutrient composition is as follows: dry matter (DM), crude protein (CP), lipids and crude fibers (CF) had been analyzed according to the instructions of TCVN 4326:2001, TCVN 4328:2007, TCVN 4331:2001 and TCVN 4329:2007. Feed formulations and nutrient compositions of the basal diets are presented in table 1.

2.2. Data collection

On a fixed day every week, the body weight of the chickens was weighed by an electronic balance (±0.01g) from birth to 5w of age. From 6 to 18w of age, a mechanical balance (±5g) was used. Differences between final and initial weights were used to compute individual ADG.

The measures on carcass traits were based on 18 F_1(Ho×LP) chickens (9 females, 9 males). The F_1(Ho×LP) chickens were slaughtered at 18w of age. The hot carcass with head (HCW) was weighed after slaughter. The killing out percentage (KOP) was calculated as a ratio of hot carcass to live weights.

The samples of thigh and breast meat from the 18 mentioned chickens were removed from left half-carcass immediately after slaughter. Each slice was weighed and put individually in a tight plastic bag. Two slices were stored at 4°C until 1 day post mortem for meat quality analysis and the last one was stored at -50°C until the analysis of meat chemical composition.

The MQ was assessed through measurements of pH, color C.I.E (L*: lightness, a*: redness and b*: yellowness), drip loss percentage (DL), cooking loss percentage (CL) and Warner-Bratzler shear force (SF). The pH value at 15min post mortem (pH15) was measured on thigh meat and breast meat in the slaughter place. The other MQ traits were determined in the laboratory at 24h post mortem. The pH, meat color and shear force were measured using a

portable pH-meter (Testo 230 with an electrode type 03 pH, Germany), a colorimeter (Minolta CR-410, Japan) with settings of illuminant D65 and 2° standard observer and a Shear force Warner Bratzler 2000D (USA) respectively.

The chemical composition of the meat was assessed by measuring DM, CP, lipids and ash contents. Only one sample of thigh and breast meat was taken from each animal but the analyses were repeated 3 times. The chemical composition of the meat were determined in the Central Laboratory of the FAS, VNUA and chemical composition is as follows: including DM had been analyzed according to the instructions of TCVN 8135:2009, CP had been analyzed according to TCVN 8134:2009, lipids had been analyzed according to TCVN 8136:2009 and ash contents (AC) had been analyzed according to TCVN 7142:2002.

2.3. Statistical analysis

Data on the GP, CC, MQ, and chemical compositions were analyzed as a randomized complete block design with dietary treatment as the experimental unit and blocks based on sex. ANOVA was generated using the GLM procedure of SAS software (SAS, 1989), with dietary treatment (T) as the main effect in the model. A linear model including the fixed effects of T, sex (S) and interaction T*S is presented in the statistical model: $Y_{ijk} = \mu + T_i + S_j + T_i*S_j + e_{ijk}$. *Where, Y_{ijk}=Parameters of the GP, CC, MQ of F_1(Ho×LP) chickens; μ=overall mean; T_i=fixed effect of treatments i^{th} (0, 0.5, 1); S_j=fixed effect of sex j^{th} (female, male); T_i*S_j=interaction between T and S; e_{ijk}=residual errors*. The means of the various diets and of both sexes were compared using Tukey's test. A significant difference was considered when the P<0.05.

3. RESULTS

The BW and ADG of F_1(Ho×LP) chickens according to the treatments are shown in Table 2 and Table 3. From birth to week 5 of age, increasing the levels of CTM in the diets did not affect the BW and ADG of F_1(Ho×LP) chickens. For period of 6-18w of age, significant linear increases in the BW, ADG were observed in chickens fed CTM diets compared with those fed the control diet. The chickens fed a diet supplemented with CTM grew faster than those in the control group. When CTM in basal diet increased to 0.5-1 mg/kg feed, the BW, ADG decreased (P>0.05).

Table 2. Effect of CTM on BW (g, Mean±SD)

Age	Control		0.5mg CTM		1mg CTM	
1D	34	40.10±1.80	31	40.27±1.83	30	40.34±1.70
1	34	80.59±10.43	31	79.03±12.74	30	77.33±12.58
2	34	159.41±21.59	31	167.10±32.17	30	160.33±21.09
3	34	250.88±40.93	31	257.42±67.13	30	238.00±43.18
4	34	351.18±51.92	31	374.84±88.24	30	356.00±57.15
5	34	476.47±64.71	31	486.77±103.58	30	448.00±89.80
6	34	608.82^{ab}±94.83	31	634.84^{a}±139.14	30	570.33^{b}±103.27
7	34	680.00^{b}±109.52	31	781.29^{a}±149.64	30	660.33^{b}±113.15
8	34	924.56^{b}±167.79	31	1064.03^{a}±177.13	30	912.67^{b}±168.37
9	34	1169.12^{b}±245.99	31	1346.77^{a}±240.30	30	1165.00^{b}±242.63
10	34	1398.53^{b}±283.10	31	1607.10^{a}±273.57	30	1445.33b±268.22
11	34	1611.65^{b}±302.94	31	1854.19^{a}±282.66	30	1624.33^{b}±302.86
12	32	1821.25^{b}±371.08	31	2059.35^{a}±349.71	30	1877.00^{b}±332.92
13	32	2018.44^{c}±393.78	30	2404.67^{a}±359.13	30	2151.67^{b}±393.28
14	32	2209.69^{b}±431.32	30	2536.00^{a}±397.87	30	2290.33^{b}±443.08
15	32	2360.94^{b}±481.22	29	2587.93^{a}±467.45	30	2393.33^{b}±463.07
16	32	2520.63^{b}±477.12	29	2727.59^{a}±486.90	30	2573.33^{b}±457.08
17	31	2596.77^{b}±459.52	29	2825.86^{a}±514.89	30	2688.33^{ab}±475.01
18	31	2754.84^{b}±450.81	29	2951.72^{a}±508.41	30	2870.00^{ab}±503.88

Note: Values in each column of each treatment with different superscripts are significantly different (P<0.05).

Table 3. Effect of CTM on ADG (g/day, Mean±SD)

Age	Control		0.5mg CTM		1mg CTM	
1	34	5,78±1,50	31	5,54±1,80	30	5,28±1,78
2	34	11,26±2,96	31	12,58±3,71	30	11,86±2,35
3	34	13,07±6,45	31	12,90±5,94	30	11,10±4,58
4	34	15,11±5,45	31	16,77±6,22	30	16,86±6,30
5	34	17,90±6,19	31	15,99±6,87	30	15,13±8,20
6	34	18,91±9,96	31	22,05±12,32	30	17,48±7,77
7	34	10,17^{b}±6,57	31	20,92^{a}±10,13	30	12,86^{b}±8,36
8	34	34,94±12,86	31	40,39±13,32	30	36,05±12,36
9	34	34,94±12,86	31	40,39±13,32	30	36,05±12,36
10	34	32,77^{b}±13,27	31	37,19^{ab}±14,88	30	40,05a±14,48
11	34	30,45^{b}±10,10	31	36,67^{a}±8,69	30	25,57c±9,27
12	32	29,93^{b}±11,46	31	29,31^{b}±15,00	30	36,09^{a}±12,94
13	32	28,17^{b}±10,84	31	45,62^{a}±13,53	30	40,69^{a}±12,63
14	32	27,32^{a}±9,79	30	19,90^{b}±12,03	30	24,73^{ab}±11,58
15	32	23,13±10,38	30	18,51±9,03	30	22,80±28,83
16	32	23,55±9,36	29	22,80±9,48	30	26,60±9,90
17	31	14,93^{b}±6,80	29	20,75^{a}±7,11	30	18,26^{ab}±10,73
18	31	23,33^{ab}±9,73	29	19,31^{b}±9,04	30	26,85^{a}±11,87

However, increasing the levels of CTM in the diets did not affect any of the parameters of the carcass characteristics of F_1(Ho×LP) chickens (Table 4)

Table 4. Effect of CTM on CC (n=6)

Variables	Cont.	0.5mgCTM	1mgCTM	SEM	P
SLW (kg)	3.03	2.85	2.95	0.10	0.472
HCW (kg)	2.27	2.17	2.18	0.07	0.577
KOP (%)	74.52	75.97	73.58	0.97	0.254
TMW (g)	486.97	442.07	443.77	17.23	0.157
TMP (%)	23.15	22.82	22.03	0.58	0.396
BMW (g)	331.40	299.60	294.40	16.38	0.263
BMP (%)	16.38	15.60	15.04	0.82	0.529

Supplemental levels of CTM in the diets did not affecte all the traits of meat quality, except CL24 of thigh meat and pH24 of breast meat (Table 5). The CL24 of thigh meat was the lowest in the diet with 0.5mg of supplemental CTM and the highest in the control group (P=0.002). Supplemental levels of CTM in the diets could be decreased the CL24 of thigh meat. The pH24 value of breast meat was the highest with 0.5mg of supplemental CTM and the lowest in the diet with 1mg of supplemental CTM (P=0.001). In contrast, SF of breast meat was the lowest in the diet with 0.5mg of supplemental CTM and the highest in the control group (P=0.074).

Table 5. Effect of CTM on meat quality (n=6)

Meat	Var	Cont	0.5mgCTM	1mgCTM	SEM	P
Thigh	pH15	6.35	6.21	6.15	0.06	0.108
	pH24	5.95	5.92	5.81	0.07	0.317
	L*24	52.64	55.06	54.51	1.08	0.291
	a*24	16.12	15.90	15.56	1.22	0.948
	b*24	9.34	9.61	8.05	0.59	0.174
	DL24 (%)	0.51	0.77	0.77	0.16	0.430
	CL24 (%)	32.97	21.68	29.57	1.76	0.002
	SF24 (N)	23.43	23.04	23.42	0.98	0.949
Breast	pH15	6.07	6.03	5.96	0.08	0.617
	pH24	5.73	5.88	5.52	0.05	0.001
	L*24	52.13	54.23	56.27	1.33	0.131
	a*24	9.23	9.52	9.17	0.95	0.962
	b*24	7.94	10.11	7.38	0.87	0.102
	DL24 (%)	1.21	2.00	1.79	0.49	0.520
	CL24 (%)	21.47	16.92	20.91	1.90	0.221
	SF24 (N)	23.84	20.25	22.80	1.02	0.074

The meat chemical composition was not affect by the supplementation levels of the CTM in the diets, except ash of thigh meat (Table 6).

Table 6. Effect CTM on chemical compositions (%)

Meat	Var	Cont	0.5mgCTM	1mgCTM	SEM	P
Thigh	DM	24.80	24.89	24.89	0.26	0.999
	CP	19.86	20.16	19.72	0.13	0.098
	Ash	0.93	1.07	0.43	0.08	0.002
	Lipid	1.25	1.23	1.24	0.03	0.951
Breast	DM	25.42	26.04	26.30	0.30	0.154
	CP	23.20	23.27	23.23	0.09	0.835
	Ash	0.18	0.29	0.26	0.04	0.144
	Lipid	1.83	1.88	2.01	0.14	0.642

4. DISCUSSION

The results indicated that CTM supplementation had a positive effect on the BW, ADG of F_1(Ho×LP) chickens period of 6-18w of age. The results of our study are consistent with previous studies Van *et al.* (2021); Yin *et al.* (2022); Razanova *et al.* (2023). Similar results were reported by Yin *et al.* (2022) who demonstrated better ADG, antioxidant status, and meat quality in broiler chickens supplemented with coated trace minerals and soybean oil. Zulqarnain *et al.* (2017) suggested that replacing inorganic with organic CTM in broiler chicken feed improves ADG, carcass characteristics, and meat quality. Kong *et al.* (2022) reported that replacing inorganic minerals with small peptide chelated minerals in broiler chickens did not negatively affect growth performance, carcass characteristics, or meat quality, but improved antioxidant status and mineral deposition. The study by Van *et al.* (2021) showed that hydroxychloride trace minerals positively impact growth, carcass quality, and gut microbiota in broiler chickens, enhancing growth performance and potentially improving meat quality. The study compared inorganic and organic trace mineral premixes in broilers feed, showing no significant difference in growth performance, carcass characteristics, or meat quality, but organic minerals improved feed efficiency (Hassan *et al.*, 2022). Feeding broilers a diet with 50% organic zinc, manganese, and copper improves growth performance, carcass characteristics, and bone quality compared to 100% inorganic forms (El-Husseiny *et al.*, 2012). The

incorporation of compound trace minerals in chicken diets significantly enhances growth performance, carcass characteristics, and meat quality. Research indicates that chelated forms of trace minerals lead to improved weight gain and feed conversion ratios in broilers. For instance, broilers receiving chelated minerals exhibited a 6.9% to 13.8% increase in live weight compared to controls (Razanova *et al.*, 2023). Additionally, the use of coated trace minerals has been shown to enhance ADG and reduce FCR, while also improving antioxidant status and meat quality. The inclusion of chelated minerals resulted in higher meat yield, particularly in thighs and shins (Razanova *et al.*, 2023). Coated minerals did not negatively impact carcass traits, maintaining quality while reducing mineral intake (Ruangpanit *et al.*, 2023). Coated trace minerals improved antioxidant status, reducing lipid peroxidation and enhancing meat quality (Yin *et al.*, 2022). The combination of trace minerals with specific fat sources, like soybean oil, further optimized meat quality (Yin *et al.*, 2022). While the benefits of trace minerals are evident, some studies suggest that the effects may vary based on the mineral source and dietary composition, indicating a need for tailored supplementation strategies.

5. CONCLUSION

Supplementation of CTM in the diet of chickens led to increases in the BW, ADG, but no effects were found on the carcass traits of F_1(Ho×LP) chickens. An addition of 0.5-1mg of CTM/kg of feed is a recommended dose to improve the production performance and meat quality of chickens. These results suggest that the use of CTM could improve the growth traits of chickens under tropical environmental conditions.

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EFFECT OF THE DIETARY SUPPLEMENTATION WITH CHESTNUT WOOD TANNIN EXTRACTS ON INTESTINAL MICROBES AND MORPHOLOGY IN CROSSBRED F_1(HO×LP) CHICKENS

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ABSTRACT

This study aimed to evaluate the effects of dietary supplementation with chestnut wood tannin extracts on the intestinal microbes and morphology in crossbred F_1(Ho×LP) chickens. This study was conducted at experimental farm of Faculty of Animal Science, VNUA from Oct 2022 to Jan 2023. A total of 120 F_1(Ho×LP) chickens (60 females, 60 males) of 1 day of age were identified individually by the number on their legs and randomly allocated into one of four dietary treatments, including T3 (basal diet+0.3g tannins/kg feed), T5 (basal diet+0.5g tannins/kg feed), PC (basal diet+1g Tylosin/kg feed) and NC (basal diet). The results showed that significant linear decreases in the *E. coli*, *Salmonella* ssp. and total anaerobic bacteria were observed in chickens fed tannin diets compared with those fed the negative control diet. The HV and WV in the jejunum, ileum, and cecum were similar among treatments (P>0.05). However, the HV and WV in the duodenum were significantly different (P<0.05). This suggests that addition of 0.5g of chestnut wood tannin extracts/kg of feed is a recommended dose to improve the influence intestinal health and microbes in chickens.

Keywords: *Chicken, intestinal microbes, morphology of intestine, tannin.*

1. INTRODUCTION

In the rapidly evolving poultry industry, maintaining optimal gut health is critical for ensuring efficient growth, disease resistance, and overall performance of chickens. The intestinal tract is not only the primary site for nutrient absorption but also a complex ecosystem where a balanced microbial population is essential for poultry's health and productivity. Disruptions in gut microbes can lead to poor nutrient utilization, compromised immune function, and increased susceptibility to pathogens, all of which negatively impact poultry production. Consequently, there is growing interest in exploring natural feed additives that can enhance gut health, modulate microbial populations, and support intestinal integrity without the negative side effects associated with antibiotic growth promoters.

Tannins, a class of polyphenolic compounds naturally found in various plant sources, have emerged as promising feed additives in animal nutrition. Known for their antioxidant, antimicrobial, and anti-inflammatory properties, tannins can influence gut health by altering microbial populations and improving intestinal morphology. Chestnut wood tannin extracts, in particular, have gained attention due to their ability to bind proteins, inhibit the growth of harmful bacteria, and improve nutrient absorption by protecting the gut lining. Unlike synthetic additives, chestnut tannins are natural and sustainable, making them attractive options in the context of modern, antibiotic-free poultry farming.

Several studies have investigated the effects of dietary supplementation with chestnut wood tannin extracts on the intestinal microbes and morphology of the intestines in chickens. Liu *et al.* (2018) found

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that chestnut tannins could improve intestinal morphology, cytokine expression, and antioxidant activities in broilers. Additionally, research by various authors has explored the impact of tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbes in weaning piglets and broiler chickens (Wang *et al.*, 2020). Furthermore, studies have shown that supplementing pigs' diet with chestnut wood extract rich in tannins had no significant effect on growth rate or carcass traits (Hassan *et al.*, 2020). Moreover, research has demonstrated the beneficial effects of water-soluble chestnut tannin extract on chicken small intestinal epithelial cell culture (Brus *et al.*, 2018). Other studies have investigated the effects of Galla chinensis extract on the growth performance and carcass traits of broiler chickens, suggesting that dietary supplementation with chestnut tannins may alter the intestinal microbes of chickens (Yin *et al.*, 2022). Additionally, the dietary application of tannins, including chestnut wood extract, has been shown to increase intestinal dry matter contents in chickens and impact intestinal morphology (Choi and Kim, 2020). Overall, these studies highlight the potential benefits of dietary supplementation with chestnut wood tannin extracts on the intestinal microbes and morphology of the intestines in chickens. Further research is needed to fully understand the mechanisms underlying these effects and to optimize the use of tannins as natural feed additives in poultry production (Mahfuz *et al.*, 2021; Redondo *et al.*, 2021).

The aim of present study was to investigate the effects of dietary supplementation with chestnut wood tannin extracts on the intestinal microbes, the hight and width of mucosa villi in crossbred F_1(Ho×LP) chickens.

2. MATERIALS AND METHODS

2.1. Experimental design, animals and diets

The experiment was conducted on 120 F_1(Ho×LP) chickens (60 females, 60 males) of 1 day of age at experimental farm of Faculty of Animal Science, VNUA from Oct 2022 to Jan 2023. The chickens were identified individually by the number on their legs and randomly allocated into one of 4 dietary treatments, including T3 (basal diet+0.3g tannins/kg feed), T5 (basal diet+0.5g tannins/kg feed), PC (positive control: basal diet+1g Tylosin/kg feed) and NC (negative control: basal diet). The powder blend used in this experiment contained 75% tannins extracted from Chestnut wood (*Castanea sativa*) by zhydrolyzable ellagitannins using hot water.

There were 10 replicates for each of four treatments with 30 chickens (15 females, 15 males) in each treatment. The chickens were kept in cage (3 chickens per cage), with free access to feed and water. From birth to week 4 of age, the chichkens were kept under a heat lamp and fed the diet with 22.14% crude protein (CP), 3027 kcal/kg metabolizable energy (ME). For period of 5-15w of age, the chickens were fed the diet with 18.10% CP, 3000 kcal/kg ME.

2.2. Data collection

2.2.1. Intestinal microbes

Cloacal swab samples and fecal samples were collected during the experiment period form 2, 8 and 15w of age according to Vietnamese Standards (TCVN 10782:2015 (ISO 13307:2013). At the end of experiments (15w of age), samples (about 10g) were collected from the ileum and cecum contents and transported into a 250ml Erlenmayer flask containing 90ml of sterile peptone saline solution (0.1% peptone+0.85% NaCl). All samples were be transported on ice and stored at 4°C until analysis.

The 1g from each sample was homogenized in 9ml sterile water and serial dilutions up to 10^5 were prepared. A volume of 100µl from the concentration of 10^{-3}, 10^{-4} and 10^{-5} was separately spread on plates

containing Macconkey agar for determining of *E. coli, Salmonella*, and Wilkins-Chalgren agar to identify total anearobic bacteria (Xia *et al.*, 2004; Sheiha *et al.*,. 2020). All the plates were incubated aerobically or anaerobically as appropriate at 37°C for 24-48h. To identify *E. coli* biochemical assays of indole, methyl red, Voges-Proskauer and citrate reactions were done (Cowan, 1985). *Salmonella* spp. were be counted using S.S. agar (Oxide CM 99). Black colonies on S.S. agar, which are typical colonies of *Salmonella* spp., were inoculated into Kligler Iron Agar slants and incubated aerobically at 35±2°C for 24-48h. Salmonella are lactose-negative, but they ferment glucose with gas and H_2S production. Bacterial population were reported as logarithmic number of bacteria/g of sample.

2.2.2. Morphology of small intestine and cecum

Samples are collected from four segments: duodenum, jejunum, ileum, and cecum and fixed with a 10% neutral formalin solution for 24-48h. After the paraffin embedding process, the specimens are cut to a thickness of 2-5μm then stained with hematoxylin-eosin (HE). The morphology of the intestinal epithelial cells is observed under a microscope, while the height and width of the intestinal villi are measured using Liteview Software. Two straight and intact villi from each segment are measured from tip to base. The hight of the villi (HV) was measured from the tip of the villus to its base (where the intestinal galnds begin). The width of the villi (WV) is determined as the distance between the outer edges of opposing epithelial cells, measured along a line passing through the longitudinal midpoint of the villi (Wang and Peng, 2008).

2.3. Statistical analysis

A linear model including the fixed effects of treatment (T), week of age (W), and interaction between these factors is presented in the statistical model below: $Y_{ijk} = \mu + T_i + W_j + T_i*W_j + e_{ijk}$. *Where, Y_{ijk}=Parameters of the intestinal microbes of F_1(Ho×LP) chickens; μ=overall mean; T_i=fixed effect of treatments i^{th} (T3, T5, PC, NC); W_j=fixed effect of week of age j^{th} (2, 8, 15); T_i*W_j=interaction between T and W; e_{ijk}=residual errors*. A linear model including the fixed effects of T, sex (S) and interaction between these factors is presented in the statistical model below: $Y_{ijk} = \mu + T_i + S_j + T_i*S_j + e_{ijk}$. *Where, Y_{ijk}=Parameters of the morphology of the intestine of HLP chickens; μ=overall mean; T_i=fixed effect of treatments i^{th} (T3, T5, PC, NC); S_j=fixed effect of sex j^{th} (female and male); T_i*S_j=interaction between T and S; e_{ijk}=residual errors*. The pairwise comparison of least square means was made using Tukey's test. Statistical parameters are number of observation (n), least square mean (LSM) and SE. A significant difference was considered when P<0.05. The data were analyzed using the linear model (lm) command of R.4.2.2 (Team, 2022) to identify significant sources of variation.

3. RESULTS

Table 1. LSM according to T, W, T*W of IM

Item		n	∑bacteria	*E. coli*	*Salmonella ssp*
T	T3	21	7.82^{a}	5.00^{a}	2.82^{b}
	T5	18	7.74^{b}	4.92^{b}	2.76^{b}
	PC	21	7.71^{b}	4.73^{c}	2.54^{c}
	NC	18	7.85^{a}	5.00^{a}	2.97^{a}
W	2	24	7.80^{a}	4.96^{a}	2.75^{b}
	8	18	7.84^{a}	4.93^{a}	2.86^{a}
	15	36	7.72^{b}	4.84^{b}	2.71^{b}
T*W	T3*2	9	7.86^{ab}	5.03^{a}	2.86^{ab}
	T3*8	3	7.84^{abc}	5.03^{ab}	2.91^{ab}
	T3*15	9	7.76^{bcd}	4.93^{bc}	2.69^{c}
	T5*2	6	7.79^{abc}	4.96^{abc}	2.79^{abc}
	T5*8	3	7.83^{abc}	4.94^{abcd}	2.84^{abc}
	T5*15	9	7.59^{e}	4.85^{cde}	2.66^{d}
	PC*2	6	7.71^{cde}	4.81^{de}	2.34^{e}
	PC*8	6	7.77^{abcd}	4.76^{e}	2.73^{bc}
	PC*15	9	7.65^{de}	4.61^{f}	2.54
	NC*2	3	7.77^{abcd}	5.04^{ab}	3.00^{a}
	NC*8	6	7.91^{a}	4.99^{ab}	2.96^{a}
	NC*15	9	7.88^{ab}	4.96^{ab}	2.95^{a}
SE			0.03	0.02	0.03
P	T		***	***	***
	W		***	***	***
	T*W		***	***	***
R^2			0.67	0.83	0.82

Note: Values in each column of each treat with different superscripts are significantly different (P<0.05)

The intestinal microbes (IM) of F_1(Ho×LP) chickens according to the T, W and T*W interaction are shown in Table 1. The *E. coli, Salmonella* ssp. and total anaerobic bacteria number were significantly different among the T, W and T*W interaction (P<0.001). Significant linear decreases in the *Escherichia coli, Salmonella* ssp. and total anaerobic bacteria concentration were observed in chickens fed tannin diets compared with those fed the negative control diet. When tannin in basal diet increased 0.3-0.5 g/kg feed, the *E. coli*, *Salmonella* ssp. and total anaerobic bacteria decreased.

Table 2. LSM according to T, S and T*S of MI

Item	n	Duodenum		Jejunum		Ileum		Cecum	
		HV	WV	HV	WV	HV	WV	HV	WV
T3	15	1064^{ab}	306^{a}	1287	212	1173	205	731	226
T5	18	891^{b}	175^{b}	1005	228	1083	271	773	249
PC	17	1159^{a}	267^{a}	1251	218	1362	226	796	231
NC	19	1177^{a}	220^{ab}	1150	249	1336	221	842	222
F	33	1183^{a}	260	1076^{b}	234	1212	233	827	225
M	36	962^{b}	223	1270^{a}	219	1265	228	744	240
T3*F	6	1045^{abc}	345	1199	194	998^{ab}	196	1014^{a}	274
T5*F	9	1056^{abc}	171	938	188	1262^{ab}	298	656^{ab}	226
PC*F	9	1402^{a}	319	1148	278	1367^{ab}	227	832^{ab}	182
NC*F	9	1230^{ab}	206	1020	275	1221^{ab}	211	807^{ab}	217
T3*M	9	1083^{abc}	267	1374	229	1348^{ab}	213	449^{b}	178
T5*M	9	726^{c}	179	1073	267	904^{b}	243	890^{ab}	273
PC*M	8	916^{bc}	214	1354	158	1358^{ab}	226	760^{ab}	281
NC*M	10	1125^{abc}	234	1280	222	1450^{a}	232	877^{ab}	227
SEM		91.61	28.17	97.21	24.54	98.91	33.49	89.62	35.36
T		*	**	ns	ns	ns	ns	ns	ns
S		**	ns	*	ns	ns	ns	ns	ns
T*S		ns	ns	ns	**	*	ns	**	ns
R^2		0.29	0.28	0.17	0.20	0.23	0.06	0.25	0.10

Note: Values in each column of each treat with different superscripts are significantly different (P<0.05)

4. DISCUSSION

It was found that increasing tannin level supplementing to the diet leaded to decrease IM of F_1(Ho×LP) chickens. However, in the present study, tannin supplementation did not affect HV and WV in the jejunum, ileum, and cecum of F_1(Ho×LP) chickens. This finding is in agreement with Jamróz *et al.* (2009); Liu *et al.* (2018). Jamróz *et al.* (2009) confirmed that higher doses of tannins significantly reduced the number of *Escherichia coli* and coliform bacteria in small intestine of 28 day of age chickens; in other microorganisms great variability of microbial populations in small intestine and colon were observed. The histologies of jejunal walls in chickens of control, 250 and 500 mg/kg groups were similar (Jamróz *et al.*, 2009). The study of Liu *et al.* (2018) on broilers revealed that the populations of *Escherichia coli* and *Clostridium* in the jejunum and caecum of broilers in the control group were higher than those in the normal and tannin supplementation with 2 g/kg groups.

Chestnut wood tannin extracts have shown beneficial effects on intestinal health and performance in chickens. Supplementation with 500 mg/kg of chestnut tannins improved intestinal morphology, increasing villus height and area (Perić *et al.*, 2022). Lower doses (250-500 mg/kg) had no significant impact on BW or FCR, while higher doses (1000 mg/kg) reduced growth performance (Jamróz *et al.*, 2009). Chestnut tannins at 2 g/kg improved growth performance, intestinal barrier function, and antioxidant capacity in heat-stressed broilers (Liu *et al.*, 2018). *In vitro* studies on chicken intestinal epithelial cells demonstrated that water-soluble chestnut tannins stimulated cell proliferation, increased antioxidative potential, and showed no genotoxicity at concentrations up to 0.1% (Brus *et al.*, 2018). Additionally, chestnut tannins reduced harmful bacteria like *E. coli* in the intestine (Jamróz *et al.*, 2009; Liu *et al.*, 2018). These findings suggest that chestnut tannin extracts can positively influence intestinal health and microbes in chickens when used at appropriate concentrations. It is in agreement with a study of Díaz Carrasco *et al.* (2018). Chestnut tannin supplementation significantly increased villus height, villus height-to-crypt depth ratio, and villus area in broiler chickens, indicating improved intestinal morphology (Perić *et al.*, 2022). In studies, chickens receiving tannins during specific growth phases exhibited longer intestines and enhanced villi, suggesting a positive influence on intestinal development

(Buyse *et al.*, 2024). The inclusion of chestnut tannins did not detrimentally affect the gut microbiome, maintaining similar microbial community structures across different dietary treatments (Daghio *et al.*, 2024). Tannins may promote beneficial microbial populations, contributing to gut health and nutrient absorption (Pascual *et al.*, 2022).

5. CONCLUSION

Supplementation of chestnut wood tannin extracts in the diet of chickens led to decreases in the intestinal microbes, but no effects were found on the HV and WV in the jejunum, ileum and cecum of F_1(Ho×LP) chickens. An addition of 0.5g of chestnut wood tannin extracts/kg of feed is a recommended dose to improve the influence intestinal health and microbes in chickens.

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SOYBEAN MEAL AS LOW-COST NUTRIENT SOURCE FOR PROBIOTICS BIOMASS PRODUCTION TO APPLYING IN THE *LITOPENAEUS VANNMEI* CULTURE

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ABSTRACT

To reduce the cost of applying probiotics in shrimp culture, the expensive commercial culture medium should be replaced with available low-cost substrates in biomass production. In this study, the soybean meal (SBM, 1% w/v) was proved to have a prebiotic index of 2.15-4.0 and was able to be used as sole nutrient source to enhance the growth of *Bacillus subtilis* and *Lactobacillus acidophilus* comparable to the commercial ones. In solid-state fermentation with SBM, the probiotics could reach a high density of (80±5)×10^{9}CFU/g and degraded 81.52±1.04% of the trypsin inhibitor, thus minimizing the indigest. The supplement of this fermented product at 10 g/kg diet increased the growth and phenoloxidase activity of the *Litopennaeus vannamei*, hence improving the shrimp survival rate when challenged with *Vibrio parahaemolyticus*. Therefore, SBM in the production of probiotics biomass for the application in shrimp could be effectively used.

Keywords: *Soybean meal, biomass production, prebiotic index, growth performance, Litopenaeus vannamei.*

1. INTRODUCTION

In aquaculture, with the current trend of developing aquaculture sustainability, there is a huge demand for the production of probiotics biomass to replace the use of several aqua-chemicals, particularly antibiotics, and pesticides (Pandey *et al.*, 2015). *Lactobacillus* and *Bacillus* strains are the most commonly used probiotics in aquaculture applications (Śliżewska & Chlebicz-Wójcik, 2020). At the laboratory scale, rich nutrient media such as the de Man, Rogosa and Sharpe (MRS) or Meat peptone (MP) are often required for the growth of these probiotics, however, their high price makes it inadequate for large-scale biomass production. To lower process costs, attention has been paid to substitutes of carbon and nitrogen sources in culture media with low-cost alternative agriculture by-products. Soybean meal (SBM) is an agro-residue of the soybean oil extraction process which is traditionally considered as a plant-based protein source in shrimp feed. Moreover, the composition analysis revealed that SBM contains a variety of nutrient contents, including sucrose, C, N, S, P, Ca, Mg, Na 76.60, 419.32, 76.17, 3.38, 2.09, 4.24, 2.39, 14.07mg/g and especially up to 6% of dietary fiber per dried weight (Redondo-Cuenca *et al.*, 2007; Roslan *et al.*, 2021). The dietary fiber, so-called prebiotics, are non-digestible feed ingredients but can selectively enhance the growth of probiotic bacteria (Figueroa-González *et al.*, 2019). Therefore, in the present study, we aimed to test SBM as a sole nutrient source for the biomass production of the *Lactobacillus* and *Bacillus* strains to form a low-cost bio-product for dietary supplement in the *Litopannaeus vannamei* culture. An *in vivo* experiment was also performed to evaluate the effectiveness of dietary supplementing this fermented product in improving the growth, and pathogen resistance of the white-leg shrimp (*Litopannaeus vannamei*).

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2. MATERIALS AND METHODS

2.1. Probiotic strains and bacterial culture medium

Probiotic strains (*Bacillus subtilis* BSHC, *Bacillus subtilis* HM and *Lactobacillus acidophilus* LB) and pathogenic bacteria (*V. parahaemolyticus* pVPA3-1) were provided by the Institute of Biotechnology, VAST. The SBM was purchased from a local supplier in Vietnam, grounded, and sorted to the size of approximately 0.1mm diameter by using a 0.105mm sieve. The SBM was prepared by adding different amounts of SBM (w/v) into phosphate buffer (pH ~ 6.5-7.0).

2.1. Growth of probiotics in the soybean meal medium

To evaluate the effect of soybean meal medium on bacteria growth, the bacteria biomass was added to the SBM medium that contained different soybean meal concentrations (0.1, 0.3, 0.5, 0.7, and 1.0%, w/v), and incubated at 35°C, 150rpm. After 24h, the bacteria growth was estimated by measuring the bacterial optical density at 600 nm (UV-1601 Spectrophotometer, Shimadzu, Japan). The growth of *L. acidophilus* LB in the MRS medium and *B. subtilis* BSHC, *B. subtilis* HM in the MP medium served as a positive control in this experiment. Additionally, the digestive enzyme secretion (α-amylase, protease, cellulase) and anti-*Vibrio parahaemolyticus* activity of these bacteria when grown in the SBM medium were measured by the agar diffusion method. (Möttönen, 1970).

2.3. Prebiotic potential of soybean meal

The prebiotic index (I_{preb}) of SBM was determined following the method of Figueroa-González *et al.* (2019). Three probiotic strains, including *B. subtilis* BSHC, *B. subtilis* HM, and *L. acidophilus* LB, were used to assess the prebiotic potential of SBM. The prebiotic index was calculated based on the ratio of probiotic growth in an SBM medium (phosphate buffer saline+1% SBM) to probiotic growth in a control carbohydrate medium (phosphate buffer saline+1% glucose).

2.4. Preparation of the bio-product by the solid-state fermentation of probiotics with SBM

B. subtilis BSHC and *B. subtilis* HM were grown under aerobic conditions, and *L. acidophilus* LB was cultured under anaerobic conditions in the SBM medium to reach a density of approximately 10^8CFU/ml. The multiple strains of probiotics suspension were prepared by mixing the three bacteria suspension at a ratio of 1:1:1. In the solid-state fermentation, to compare the degradation capacity of trypsin inhibitor of single and multiple strains of probiotics, each prepared bacteria suspension was separately mixed with SBM to reach a moisture content of 40%. After 48h of incubation at 35°C, the density of bacteria in the fermented product was determined to ensure the viability of the bacteria. Additionally, the residual trypsin inhibitor in SBM was measured according to the American Oil Chemists' Society (AOCS, 2017).

2.5. Feeding trial

To examine the beneficial effect of the fermented product on *L. vannamei* shrimp culture, an *in vivo* experiment was carried out at the Biotechnology Center of Ho Chi Minh City, Vietnam. The *L. vannamei* shrimp postlarvae were nursed in an indoor recirculating nursery system and fed only with a basal diet suggested by Jalali *et al.* for one month (Jalali *et al.,* 2009). When the average shrimp body weight reached approximately 1.72±0.2g, they were divided into 12 composite tanks (100 L, three tanks per treatment) with 45 shrimps per tank. The fermented product was mixed with basal diet to reach different probiotics density in the diet: Pro1=approximately 10^6CFU probiotics/kg of feed; Pro2=approximately 10^5CFU probiotics/kg of feed; Pro3=approximately 10^4CFU probiotics/kg of feed. The negative control group (C) was fed only by basal diet without probiotics supplements. Shrimps were fed 5% of their body weight each day, three times per day. During the 21 days of the feeding experiment, the weight gain (WG), the specific growth rate (SGR) as well as survival rate (SR) of the shrimp were monitored according to Adel *et al.* (2017).

$$WG\ (\%) = \{(\text{Final weight} - \text{initial weight})/\text{initial weight}\} \times 100\%$$

$$SGR\ (\%) = \{(\ln \text{ of final weight} - \ln \text{ of initial weight})/\text{time of cultivation}\} \times 100\%$$

$$SR\ (\%) = (\text{Final number of live shrimp}/\ \text{Number of initial shrimp}) \times 100\%$$

Additionally, shrimp body weight and feed consumption in each treatment were monitored to estimate the feed conversion ratio (FCR) based on the following equation (Chowdhury & Roy, 2020):

$$FCR = \text{Feed is given}/\ \text{shrimp weight gain}$$

2.6. AHPND bacterial challenge test

After 21 days, the shrimps in each probiotics-fed and negative control group (21 shrimps per group) were challenged with *V. parahaemolyticus* pVPA3-1 at the LD_{50} dose. Afterward, the shrimps in each corresponding treatment were continuously cultured with the same previous diet. The protective efficacy of probiotics against pathogenic infection was determined by calculating the shrimp survival rate after 14 days. The hepatopancreas was excised and stained with Uranyl acetate (Sigma-Aldrich, UK) to detect the severity of the AHPND in the hepatopancreas of dead shrimp samples using a light microscope (Olympus CKX41, Tokyo, Japan). The relative percent of survival (RPS) in each group was calculated as follows *(Luan et al.,* 2021):

$$RPS\ (\%) = 100 \times \left(1 - \frac{\text{average cumulative mortality rate of the group fed with probiotics}}{\text{average mortality rate of the negative control}}\right)$$

2.7. Statistical analysis

All experiments were carried out in triplicate. The values were presented as the Mean±SD (n=3). The statistical significance was determined using Student's t-test, $P<0.05$ was considered as an indicator of statistical significance between the two tested groups.

3. RESULTS

3.1. Prebiotic index and activity score of soybean meal

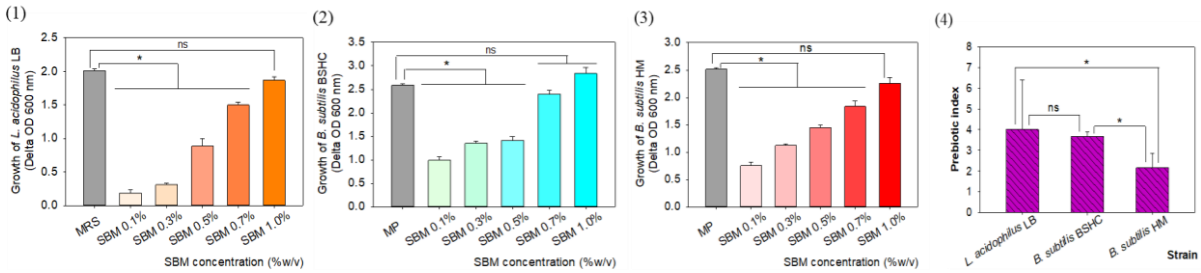

Figure 1. Effect of SBM concentration on the growth of *L. acidophilus* LB

*(1), B. subtilis BSHC (2), and B. subtilis HM (3) and prebiotic index of SBM when tested with the three probiotics (4). Values were presented as the mean ± SD (n=3). In Figure 1–1, 2, and 3, *P<0.05 versus the control (commercial medium) samples. In Figure 1–4, *P<0.05 between two tested samples. ns means that it is not significant.*

In the present study, when SBM was supplied as a solely nutrient source in probiotics culture, the growth of the *B. subtilis* BSHC, *B. subtilis* HM, and *L. acidophilus* LB gradually increased with increasing SBM concentration and reached their highest bacterial optical density in the medium containing 1.0% SBM (w/v). The growth of the probiotics in this SBM medium was comparable to that in commercial ones (Figures 1-1, 2 and 3). Moreover, the bacterial density of probiotics that grew in the SBM medium (1% w/v) was compared to that in the glucose medium (1% w/v) to determine the prebiotic index (I_{preb}) of SBM. The results presented in Figure 1-4 showed that SBM had the highest I_{preb} of 4.0±2.4 when tested with *L. acidophilus* LB, followed by *B. subtilis* BSHC, and *B. subtilis* HM strains with values of 3.68±0.2, and 2.15±0.7, respectively. A study by Figueroa-González et al showed that the I_{preb} of greater than 1 could indicate the

positive effect of the tested prebiotic on the growth of probiotics (Figueroa-González *et al.,* 2019). Additionally, when grown in the SBM medium, these probiotics could produce all three types of digestive enzymes, including α-amylase, cellulase, and protease, which are indicated via the high hydrolytic zones (Table 1). The probiotics also were able to inhibit the growth of *V. parahaemolyticus* pathogen with an inhibition zone of approximately 18.5±1.6-32.5±0.8mm. Therefore, it is suggested that the SBM not only acted as a nutrient source but also as a prebiotic provider in supporting the growth of these probiotics.

Table 1. The enzymes secretion and anti-*V.parahaemolyticus* activity of probiotics grew in SBM medium

Strain	Diameter of hydrolytic zones (mm)			*V. parahaemolyticus* inhibition zone (mm)
	Starch	*CMC*	*Casein*	
B. subtilis BSHC	19 ± 1.2	28 ± 0.8	34 ± 1.6	25 ± 1.2
B. subtilis HM	20 ± 0.8	30 ± 1.6	34 ± 2.4	32.5 ± 0.8
L. acidophilus LB	18 ± 1.6	27 ± 0.8	29 ± 1.6	18.5 ± 1.6

3.2. Preparation of the bio-product by the solid-state fermentation of probiotics with SBM

To replace the use of an expensive commercial culture medium with an alternative low-cost substrate like soybean meal in the production of probiotics biomass, in the solid-state fermentation experiment, the starter bacterial culture was prepared by solely growing the three probiotics in the SBM (1% w/v) medium to reach a bacterial density of approximately 10^5CFU/g. The bacterial mixture was then mixed with SBM and continued to ferment for 48h. Besides the nutrient contents, the SBM also contains several undesirable antinutritional factors such as trypsin inhibitors (TI), which can form competitive bindings that reduce the proteolytic activity of chymotrypsin and trypsin enzymes in not only poultry, and livestock but also in aquatic animals such as shrimp (Senphan *et al.,* 2015). TI is a highly stable compound that is difficult to degrade by heat treatment (Vanga *et al.,* 2018). However, during the fermentation process, bacteria can utilize the accessible nutrient of SBM to support their growth and concurrently secrete exogenous enzymes to degrade the anti-nutritional factors. In this study, different strains of probiotics were tested for their trypsin inhibitor removal activity. The results depicted in table 2 showed that the multiple-strain culture could degrade TI more effectively than the single strain. A TI degradation efficiency of 81.52±1.04% was detected by the fermentation of SBM with the mixed culture of *B. subtilis* BSHC, *B. subtilis* HM, and *L. acidophilus* LB, which was higher than the value of 64-75% in single strain fermentation. Additionally, a high density of probiotics of (80±5)×10^9CFU/g was measured in the mixed-strain fermented SBM. Therefore, the fermentation of SBM with multiple strains of probiotics was selected for evaluation of their effect on the *L. vannamei* growth in an *in vivo* experiment.

Table 2. Change in probiotics density (CFU/g) and trypsin inhibitor content (TUI/g) of fermented SBM

Strain	Probiotics density (CFU/g)		Trypsin inhibitor content (TUI/g)	
	Before fermentation	*After fermentation*	*Before fermentation*	*After fermentation*
B. subtilis BSHC	(63±12)×10^5	(89±8)×10^8	38.95±0.64	9.79±0.44
B. subtilis HM	(58±39)×10^5	(53±10)×10^8	40.72±0.53	14.28±1.16
L. acidophilus LB	(55±14)×10^5	(28±3)×10^7	42.03±0.27	11.62±1.39
Multiple-strain	(68±2)×10^5	(80±5)×10^9	41.12±0.41	7.60±0.44

3.3. Effect of the fermented product on the growth performance of *L. vannamei*

The results obtained from the *in vivo* experiment showed that the shrimp survival rate slightly decreased to 93.33% in the Pro1 diet, but the reduction was insignificant compared to the negative control. The feed conversion rate was stable in a range of 2.59-

2.92 among the experimental groups. Especially, compared to the negative control, a significant increase in the growth performance of *L. vannamei* was recorded in the probiotic-fed groups after 21 days of culture. Shrimps fed with the Pro1 diet achieved the highest weight gain of 83.46±4.71% and a specific growth rate of 2.81±0.12, which were higher than the Pro2 and Pro3 groups (Table 3). It is worth mentioning that these beneficial effects were achieved after a shorter culture time of 2-3 times than in other studies. For instance, the improvement in total weight of shrimp fed with (5g of KOME+10^8CFU of probiotic)/kg of feed) was observed after 8 weeks of culture, while a longer period of 60 days was required for a diet containing (0.4% GOS+10^8CFU of probiotic)/kg of feed to significantly increase the weight gain of shrimp compared to the control (Huynh *et al.*, 2018; Prabawati *et al.*, 2022). Therefore, the use of the fermented product at a minimum inclusion level of 10^6CFU/kg of feed in the Pro1 diet could effectively improve the growth performance of *L. vannamei* shrimp without affecting its survival rate.

Table 3. Effect of probiotics administration on the growth performance of *L. vannamei* shrimp

	Negative control	Pro3 diet	Pro2 diet	Pro1 diet
Survival rate (%)	100	97.78 ± 3.14	100	93.33
Feed conversion rate	2.59 ± 0.04	2.62 ± 0.12	2.53 ± 0.11	2.92 ± 0.31
Initial weight	1.64 ± 0.17 ^{a}	1.59 ± 0.60 ^{a}	1.79 ± 0.15 ^{a}	1.85 ± 0.21 ^{a}
Final weight	2.12 ± 0.35 ^{a}	2.24 ± 0.33 ^{b}	2.64 ± 0.15 ^{b}	3.38 ± 0.34 ^{c}
Weight gain (%)	28.14 ± 7.18 ^{a}	40.21 ± 5.77 ^{b}	48.01 ± 6.24 ^{b}	83.46 ± 4.71^c
Specific growth rate (%)	1.17 ± 0.27 ^{a}	1.61 ± 0.19 ^{b}	1.86 ± 0.20 ^{b}	2.81 ± 0.12 ^{c}

Within a row, different letters indicate statistically significant differences between the two tested groups ($p < 0.05$).

3.4. Effect of the fermented product on the *V. parahaemolyticus* resistance of *L. vannamei*

Table 2. Effect of probiotics administration on the *V. parahaemolyticus* pVPA3-1 resistance of *L. vannamei*

Diet	Mortality rate (%)	RPS (%)
Pro1	33.33±6.73^a	36.36±9.62^a
Pro2	47.62±17.82^b	18.18±25.46^b
Pro3	57.14±20.20 ^{b}	9.09±28.86^b
Negative control	57.14 ^{b}	0

After being infected with *V. parahaemolyticus*, the hepatopancreatic cells of these shrimp were attacked, causing the appearance of hepatopancreatic necrosis (Figure 2-2). As a result, the hepatopancreatic tubules were damaged and invaded by intraluminal hemocytes. Interestingly, the shrimp fed with the Pro1 diet could reduce the mortality by approximately two times compared to the control, to only 33.33±6.73%, meaning a higher survival rate was gained. The histopathological study also revealed that several clear lumen tubules were observed in the Pro1 diet-fed group, indicating this group of shrimp might have less severe hepatopancreatic degeneration compared to the negative control group (Figure 2).

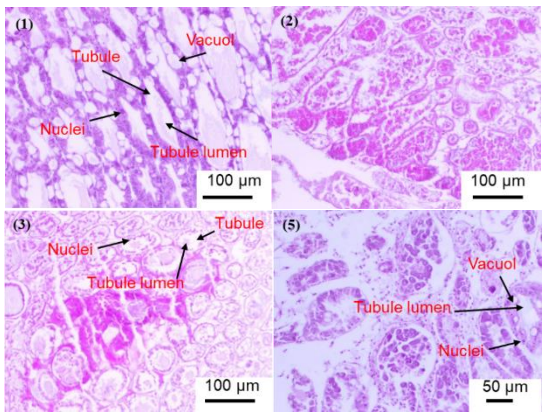

Figure 2. Histopathological changes in the hepatopancreas of healthy (1) and *V. parahaemolyticus* pVPA3-1 infected *L. vannamei* shrimps (Figure 2-2, 3, 4 and 5). (2) Hepatopancreas histology of shrimp in the control group. (3), (4) and (5) Hepatopancreas histology of shrimp fed with Pro1, Pro2, Pro3 diets.

4. CONCLUSION

In the present study, the probiotic's ability to use SBM as a main nutrient and

prebiotic source to support their growth was proved. The fermentation of these probiotics with SBM significantly degraded 81.52±1.04% of the trypsin inhibitor content. The administration of the prepared probiotics biomass at 10^6CFU/kg diet (Pro1) could quickly increase the growth performance of *L. vannamei* approximately compared to the control after 21 days of culture. Moreover, the higher *V. parahaemolyticus* pVPA3-1 resistance was also observed in the Pro1-fed shrimp, when challenged with the pathogen, the shrimp mortality rate significantly decreased from 57.14% in the control diet to 33.33±6.73% in the Pro1 diet. These results suggested that the use of SBM in the production of *B. subtilis* and *L. acidophilus* biomass could create a low-cost but high-quality dietary supplement for *L. vannamei* culture.

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BIOMASS CULTIVATION OPTIMIZATION OF *LACTOBACILLUS PLANTARUM* N1 ISOLATED FROM HEALTHY PIG INTESTINES FOR PROBIOTIC DEVELOPMENT

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ABSTRACT

Lactobacillus plantarum N1, isolated from the intestines of healthy pigs, shows potential as a probiotic for production. This strain exhibits bile tolerance with a survival rate of over 70% after 4h in a medium containing 0.3% bile salts. Additionally, N1 is capable of inhibiting several common pathogenic microorganisms such as *Staphylococcus* sp., *Salmonella* sp., and *E. coli*. Molasses, yeast extract, and soytone were identified as key factors affecting the biomass production of N1 and were thus applied in optimization using Response Surface Methodology (RSM). The optimized fermentation medium composition includes 35 g/l molasses, 24.8 g/l yeast extract, 26.2 g/l soytone, 5 g/l CH_3COONa, 2 g/l K_2HPO_4, 1 g/l Tween 80, 0.1 g/l $MgSO_4.7H_2O$ and 0.05 g/l $MnSO_4.H_2O$, with a predicted maximum biomass of $7.78x10^9$±0.73 CFU/ml according to RSM. The fermentation was conducted at 37°C, pH 6, with an inoculation ration of 10%. Experimental results after optimizing the medium components yielded a biomass density of $7.56x10^9$ CFU/ml, which is lower than the predicted value but 1.83 times higher than that achieved with the non-optimized medium.

Keywords: *Probiotic, L. plantarum, antibacterial activity, bile salt, response surface methodology.*

1. INTRODUCTION

Adding probiotics to animal feed is recommended to stimulate appetite (Bhogoju and Nahashon, 2022), improve and establish gut microbiota balance, enhance digestion and stimulate the immune system (Perdigon *et al.*, 1999) in animals. Therefore, in animal husbandry, especially industrial farming, the use of probiotics to improve gut health and, consequently, overall health and production performance (Budiño *et al.*, 2005) in pigs is essential. Duan *et al.* (2012) suggest that supplementing probiotics containing *Lactobacillus* positively affects digestion in pigs. Among *Lactobacilli* strains, which include over 50 species, *L. plantarum* is one of the most common and versatile bacteria used in the fermentation of vegetables, meat, and dairy products as well as in animal feed supplements. The *L. plantarum* N1 strain used in this study was isolated from healthy pigs' intestines, enhancing resistance to intestinal diseases and promoting growth in pigs. The basic medium used for culturing *Lactobacillus* is MRS, which includes essential nutrients and growth-inhibiting components to prevent the growth of unwanted bacteria. However, MRS medium is insufficient to maximize the growth of certain *Lactobacillus* strains. *Lactobacilli* are fastidious bacteria requiring nutrient-rich media due to their varying abilities to metabolize nutrients like sugars, amino acids, peptides, and vitamins, depending on the strain. Consequently, the medium composition and culture conditions significantly affect the production of viable cells, biomass, and metabolites of *L. plantarum*. If a new medium for each strain or species could be created as an alternative to conventional media, it would enhance biomass production and provide economic benefits, including reduced costs, wastewater, and fermentation time. We optimized the medium using various strategies to find effective ways to increase the yield. Although one-factor-at-a-time (OFAT) approach is simple and convenient,

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OFAT involves a large number of experiments as the number of factors increases. Furthermore, this method ignores the interactions between factors (Liu *et al.*, 2010). Therefore, RSM, a statistical method, is preferred due to its efficiency. Moreover, RSM considers the effects of factors, relationships between variables, and optimal conditions (Coelho *et al.*, 2011).

2. MATERIAL AND METHODS

2.1. Bacterial strain and medium

The *L. plantarum* N1 strain (NCBI Code: PP917558), isolated from the intestines of healthy pigs, was used in this study. The stock culture was preserved at -80°C in 30% sterile glycerol (v/v). The basic medium for bacterial growth was MRS (Difco, USA), which consists of 5 g/l sodium acetate, 2 g/l K_2HPO_4, 1 g/l Tween 80, 0.1 g/l $MgSO_4 \cdot 7H_2O$, and 0.05 g/l $MnSO_4 \cdot H_2O$. The initial pH of the media was adjusted to 6.5±0.05 using 1M NaOH and 1M HCl prior to sterilization. MRS medium was used as the control (non-optimized medium) compared with the optimized medium.

2.2. Determination of cell density

A single colony was inoculated into MRS medium and cultured in liquid form until the optical density (OD) reached 0.5±0.05 at a wavelength of 600nm. A 100ml testing medium was inoculated with 10% (v/v) of the inoculum in a 250ml Erlenmeyer flask and incubated at 37°C for 24h without shaking. After cultivation without shaking, the number of bacteria in the testing media was quantified by the Conoly forming unit counting method.

2.3. Effects of pH, temperature, inoculum age, and bile salts on the bacterial strain

The initial pH, incubation temperature, inoculum age, inoculum ratio, and bile salt effects on *L. plantarum* N1 were studied to determine the optimal culture conditions. The pH was adjusted from 4.0 to 9.0 using 1M NaOH and 1M HCl prior to sterilization, and the incubation temperature was set between 22-37°C. At 0 and 4h of incubation, cell density was determined and compared in media containing 0.3% bile salt.

2.4. Antibacterial activity

The strain's resistance to four test Bacteria-*Salmonella* sp., *E. coli*, *Staphylococcu*s sp. and *Bacillus* sp., which are common intestinal pathogens in livestock, was determined using the agar diffusion method. The optimal conditions were evaluated by comparing cell density after 24h of liquid culture.

2.5. Optimization using RSM and CCD

RSM with CCD was carried out to optimize the concentrations of the medium components and estimate the effects of each variable and their interactions. Screening of independent variables using the Plackett–Burman Method with six variables were maltose, sucrose, lactose, yeast extract, soytone, and tryptone. Maltose, yeast extract, and soytone were used as independent variables. The variables were set at five different levels ($-\alpha$, -1, 0, 1, α). The medium was prepared according to the combinations of variables in the experimental runs, and all experiments were conducted under static conditions. The biomass obtained was used to establish a regression model and a second-order polynomial equation.

2.6. Fermentation of *L. plantarum* N1

The fermentation of *L. plantarum* N1 in the optimized medium and conditions was performed in a 250ml Erlenmeyer flask. The medium was inoculated with 10% (v/v) of the inoculum cultured in MRS medium for 24h. The temperature was controlled at 37°C, and the pH was maintained at 6.5. The agitation speed was set at 150rpm. MRS medium was used as a control, and the fermentation process was conducted under the same conditions. The cell density was determined after 24h.

2.7. Statistical Analysis

All experiments were repeated three times. Data are presented as Mean±SD. One-way ANOVA was used to determine the level of significant differences. Values were considered significant at $P<0.05$, and all analyses were performed using GraphPad Prism ver. 6.0. RSM was conducted by design expert 13 (Stat-Ease Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Effect of temperature and pH on the growth

Temperature is an important factor affecting the growth of *L. plantarum* N1. The results of bacterial culture in MRS medium at three temperatures of 25, 30 and 37°C for 24h showed that the highest cell density was achieved at 37°C and the lowest at 25°C. Specifically, at 37°C, the bacterial number reached 4.1x10^{9}CFU/ml, significantly higher than the 2.2x10^{9}CFU/ml at 25°C and 3.4x10^{9}CFU/ml at 40°C (Figure 1A). This demonstrates that *L. plantarum* N1 grows best at 37°C. The study by Robinson et. al. indicated that *L. acidophilus* grew optimally in 30-40°C range, which aligns with the findings for *L. plantarum* N1. Therefore, 37°C was the most suitable temperature for fermenting *L. plantarum* N1. This temperature is within the optimal range of 30-40°C for species of the genus *Lactobacillus* (Robinson, 2014). *L. plantarum* was successfully cultured at pH 3.5. However, when the pH became too acidic, growth was inhibited (Giraud *et al.*, 1991). The growth of *L. plantarum* N1 was studied within the pH range of 3.5-6.5 at 37°C. After 24h of cultivation, bacterial growth varied with pH: the maximum growth was recorded at pH 6.0 (3.9x10^{9}CFU/ml), while the lowest was observed at pH 3.5-4.5 (1x10^{9}CFU/ml), as shown in figure 1B. The optimal pH conditions for strain N1 were similar to those of *L. plantarum* 200655 (Choi *et al.*, 2021), and this pH level was used for subsequent studies.

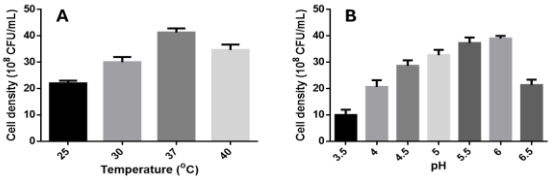

Figure 1. The effect of temperature and pH on the growth of *L. plantarum* N1

3.2. Effect of inoculum on the growth of *L. plantarum* N1

The duration of inoculum cultivation, or inoculum age, affects biomass and fermentation performance. To determine the optimal inoculum age for liquid culture to achieve the highest biomass yield, after transfer to MRS medium, the cultures were incubated statically at 37°C. Cell density was measured and recorded after 1, 2, and 3 days of incubation. Cell density increased from 1.0x10^9 to 3.4x10^{9}CFU/ml as the inoculum age increased. An inoculum age of 3 days yielded the highest cell density (3.4x10^{9}CFU/ml). With an inoculum age of 1 day, the cell density was high (1.0x10^{9}CFU/ml), similar to the fermentation conditions for *L. delbrueckii* (de Françae*t al.*, 2009). An inoculum age of 1 day was selected for inoculum production (Figure 2A). The inoculum ratio impacts bacterial biomass, a ratio that is too low can affect cultivation time, increase the risk of contamination, and result in low biomass. Conversely, a too high ratio shortens the cultivation time but can lead to rapid depletion of nutrients and the production of growth-inhibiting substances. Selecting an appropriate inoculum ratio ensures efficient fermentation, optimizes resource use, and reduces fermentation time. Inoculum ratios of *L. plantarum* N1 were investigated at 5, 10, 15 and 20% at 37°C. After 1 day, bacterial density was determined. Inoculum ratios below 5% or above 20% reduced fermentation efficiency. The 10 and 15% inoculum ratios yielded cell densities of 3.3x10^9 and 4.8x10^{9}CFU/ml, respectively. Since the difference in cell density between the 10 and 15% inoculum ratios was not statistically significant, the 10% inoculum ratio was selected (Figure 2B).

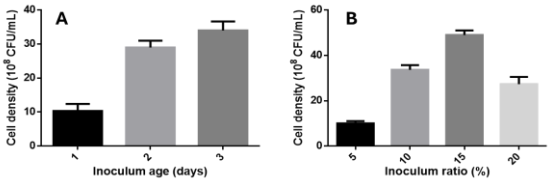

Figure 2. The effect of inoculum age (A) and ratio (B) on the cell density of *L. plantarum* N1

3.3. Bile tolerance of *L. plantarum* N1

The strain *L. plantarum* N1, after 4h of incubation in a medium with a bile salt concentration of 0.3%, exhibited a survival rate of 70%, with the final concentration of $5.06x10^9$cfu/ml after incubation. This high survival rate ensures that the probiotic microorganisms remain viable at levels $>10^7$cfu/ml. The strain's resistance to bile salts is due to its ability to produce hydrolase enzymes (taurodeoxycholic acid hydrolase and taurocholic acid hydrolase), which hydrolyze bile acids into amino acids and cholesterol, thereby reducing their toxicity and allowing the bacteria to survive in the small intestine of animals. This result is consistent with the study by Al-Saleh *et al.* (2006), which reported that during the first 3h, the growth rate of *L. acidophilus* DSM 20079 and DSM 20242 in MRS medium supplemented with 0.3% bile salt did not change significantly. However, after 5h of incubation, microbial density increased significantly and continued to rise after 24h of observation (Al-Saleh*et al.*, 2006).

3.4. Antibacterial activity of *L. plantarum* N1

L. plantarum N1 was tested for its antibacterial activity against four indicator bacteria: *Salmonella sp.*, *E. coli*, *Staphylococcus sp.*, and *Bacillus sp.*, which are commonly associated with gastrointestinal diseases in livestock (Table 1).

Table 1. Antibacterial activity of *L. plantarum* N1

Time	Inhibition Zone (mm)			
	Salmonella	*Bacillus*	*Staphylococcus*	*E. coli*
24h	13.7±0.6	11.3±1.7	13.7±0.6	16.3±0.6
48h	18.0±1.0	14.7± 0.6	19.0±2.6	18.7±0.6
72h	15.3±1.2	13.0±1.0	17.7±0.6	17.0±1.0
96h	9.7±0.6	11.3±0.6	13.7±1.5	16.3±1.5

L. plantarum N1 demonstrated antibacterial activity against all four tested bacterial strains, exhibiting a broad inhibitory spectrum against both Gram-positive and Gram-negative bacteria. The inhibition zones ranged 9.7-19mm in diameter (Table 3). The inhibitory activity of *L. plantarum* N1 against pathogenic bacteria is notably high, with inhibition zones measured as follows *Staphylococcu*s sp. 19mm, *E. coli* 18.7mm, *Salmonella* sp. 18mm, and *Bacillus* sp. 14.7mm. The results are consistent with studies on *L. acidophilus* (Mai *et al.*, 2008; Bilkova *et al.*, 2011) which also showed effective inhibition of *E. coli* causing diarrhea in humans and animals with inhibition zones greater than 16 mm. *L. plantarum* N1 has the capability to produce antibacterial compounds such as reuterin, reutericyclin, and 2-pyrrolidone-5-carboxylic acid. Additionally, it generates other inhibitory substances during its growth, including lactic acid, bacteriocin, CO_2, H_2O_2, and diacetyl. The strongest antibacterial activity was observed at 48h of incubation, with a subsequent decrease at 72 and 96h (Bilkova *et al.*, 2011).

3.5. Optimization of cultivation medium for high yield of *L. plantarum* N1 biomass

A univariate method was employed to study the effect of carbon and nitrogen sources on bacterial cell mass. The independent effects of 5 carbon types and 5 nitrogen types on bacterial density are presented in table 2.

Table 2. Effect of Carbon and Nitrogen sources on Bacterial density

Bacterial Density ($x10^8$ CFU/ml)	Carbon Source	Glucose	Sucrose	Molasses	Lactose	Maltose
		3.1	2.2	9.2	9.05	7.8
	Nitrogen Source	Peptone	Soytone	Tryptone	Yeast extract	Beef extract
		1.1	7.9	6.2	6.1	3.1

The six substrates that yielded high efficiency, molasses, lactose, yeast extract, soytone, and tryptone, were selected for further screening of independent factors affecting cell density using the Plackett-Burman method (PBM).

Table 3. Experimental design and test values for screening independent variables by PBM

Run	Maltose (g/l)	Molasses (g/l)	Lactose (g/l)	Yeast extract (g/l)	Soytone (g/l)	Tryptone (g/l)	Bacterial density (x10^{8}CFU/ml)
1	-1 (10)	-1 (10)	-1 (10)	-1 (5)	-1 (5)	-1 (5)	1,1 x 10^7
2	+1 (30)	+1 (30)	-1 (10)	-1 (5)	-1 (5)	+1 (10)	2,74 x 10^9
3	-1 (10)	-1 (10)	+1 (30)	-1 (5)	+1 (10)	+1 (10)	1,41 x 10^9
4	+1 (30)	-1 (10)	+1 (30)	+1 (10)	+1 (10)	-1 (5)	2,7 x 10^9
5	-1 (10)	+1 (30)	+1 (30)	-1 (5)	+1 (10)	+1 (10)	2,7 x 10^9
6	0 (20)	0 (20)	0 (20)	0 (7,5)	0 (7,5)	0 (7,5)	1,7 x 10^9
7	0 (20)	0 (20)	0 (20)	0 (7,5)	0 (7,5)	0 (7,5)	2,4 x 10^9
8	+1 (30)	+1 (30)	-1 (10)	+1 (10)	+1 (10)	+1 (10)	3,8 x 10^9
9	+1 (30)	-1 (10)	-1 (10)	-1 (5)	+1 (10)	-1 (5)	3,05 x 10^8
10	-1 (10)	+1 (30)	+1 (30)	+1 (10)	-1 (5)	-1 (5)	2,8x 10^9
11	+1 (30)	+1 (30)	+1 (30)	-1 (5)	-1 (5)	-1 (5)	2,6 x 10^9
12	+1 (30)	-1 (10)	+1 (30)	+1 (10)	-1 (5)	+1 (10)	3,5 x 10^7
13	-1 (10)	-1 (10)	-1 (10)	+1 (10)	-1 (5)	+1 (10)	3 x 10^8
14	-1 (10)	+1 (30)	-1 (10)	+1 (10)	+1 (10)	-1 (5)	3,5 x 10^9

The results shown in figure 3A illustrate the ranking of the impact of six factors on bacterial density. The concentration of molasses has a relatively strong correlation with biomass (correlation coefficient 0.612), while the other two factors show a weak correlation with biomass (<0.1). The factors studied include carbon sources: molasses, yeast extract, and soy protein, which are used in the optimization experiment.

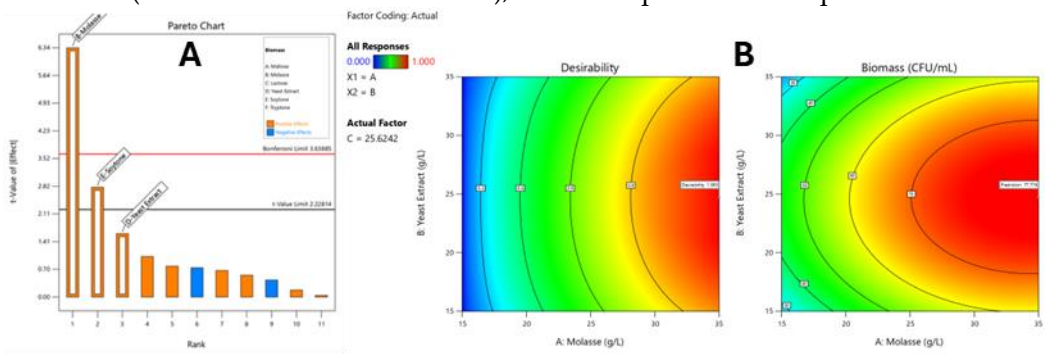

Figure 3. Optimization of biomass production

A) Ranking the impact of 6 factors on bacterial density. B) Optimization of substrate concentrations by RSM-CCD

According to RSM-CCD, the regression equation reflecting the relationship between the three factors is as follows: Y represents the cell density (10^{8}CFU/ml); X_1: the concentration of molasses (g/l); X_2: the concentration of yeast extract (g/l); X_3: the concentration of Soytone (g/l).

$$Y = -200{,}553 + 6{,}046 \times X_1 + 9{,}794 \times X_2 + 4{,}045 \times X_3 + 0{,}005 \times X_1X_2 - 0{,}004 \times X_1X_3 - 0{,}035 \times X_2X_3 - 0{,}088 \times X_1^2 - 0{,}183 \times X_2^2 - 0{,}058 \times X_3^2$$

The optimal solution will be provided by the software within the range of substrate concentrations. Figure 3B shows the maximum biomass of 7.78±0.73×10^{9}CFU/ml, corresponding to molasses, soytone and yeast extract at 35, 26.2, 24.8g/l.

The *L. plantarum* N1 was isolated from the intestines of healthy pigs and exhibited antimicrobial activity against common pathogenic strains in pigs such as *Staphylococcus* sp., *E. coli*, and *Salmonella* sp., *Staphylococcus* sp. as well as resist to bile. This

strain is classified as a biosafety microorganism suitable for use in animal husbandry. Optimizing the production of *L. plantarum* N1 biomass using inexpensive, locally available materials is a critical need. The optimal fermentation conditions are at 37°C, pH 6, with a 1-day-old inoculation ratio of 10%. In the optimized medium, the biomass of *L. plantarum* N1 reached 7.56x10^{9}CFU/ml, which is lower than the predicted value but 1.83 times higher than the non-optimized medium with 4.13x10^{9}CFU/ml, with molasses-a cost-effective, locally available raw material being used as a substrate. This indicates that *L. plantarum* N1 has a strong potential for probiotic biomass production under optimized fermentation conditions.

Table 4. Experimental design and data according to RSM-CCD of N1 biomass optimization

Run No.	Molasses (ml/l)	Soytone (ml/l)	Yeast extract (g/l)	Cell density (10^{8}CFU/ml)
1	0 (25)	0 (25)	0 (25)	50,2
2	+1 (35)	+1 (35)	+1 (35)	50,7
3	0 (25)	0 (25)	0 (25)	70,6
4	-1 (15)	-1 (15)	-1 (15)	14,8
5	-1 (15)	-1 (15)	+1 (35)	25
6	0 (25)	0 (25)	0 (25)	72,1
7	+1 (35)	-1 (15)	-1 (15)	50
8	+1 (35)	-1 (15)	+1 (35)	55,7
9	0 (25)	0 (25)	+1,682 (41,82)	60,2
10	-1 (15)	+1 (35)	+1 (35)	15,1
11	0 (25)	0 (25)	-1,682 (8,18)	48,38
12	0 (25)	0 (25)	0 (25)	75,3
13	-1 (15)	+1 (35)	-1 (15)	22
14	0 (25)	-1,682 (8,18)	0 (25)	23
15	+1 (35)	+1 (35)	-1 (15)	56
16	0 (25)	0 (25)	0 (25)	74,3
17	+1,682 (41,82)	0 (25)	0 (25)	73,6
18	-1,682 (8,18)	0 (25)	0 (25)	18,1
19	0 (25)	1,682 (41,82)	0 (25)	15
20	0 (25)	0 (25)	0 (25)	75,6

4. CONCLUSION

The combination of univariate and multivariate optimization methods in the biomass production of *L. plantarum* N1 through liquid fermentation improved biomass yield by 1.83 times compared to non-optimized conditions. The *L. plantarum* N1 strain exhibited favorable biological properties such as antibacterial activity, growth at acidic conditions, and bile tolerance.

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INFLUENCE OF MATURATION DURATION, CONCENTRATION AND TIMING OF 6-DMAP TREATMENT ON PARTHENOGENETIC BLASTOCYST PRODUCTION OF CỎ GOAT OOCYTES

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ABSTRACT

This study aimed to evaluate the effect of maturation duration, concentration and timing of 6-DMAP treatment on parthenogenetic blastocyst production of Cỏ goat oocytes. In experiment 1, the cleaved, blastocyst and hatching rates of 22 and 24h groups were higher than that of 18 and 20h groups (P<0.05). In experiment 2, matured Cỏ goat oocytes with 22h of maturation were activated for four 6-DMAP concentrations: 1, 2, 4 and 6mM. The cleaved, blastocyst and hatching rates of the 2mM group were higher than those of the 1, 4 and 6mM groups (P<0.05). In experiment 3, matured Cỏ goat oocytes were activated at 2mM with timing of 6-DMAP treatment for 2, 3, 4 and 5h. The cleavage, blastocyst, and hatching blastocyst rates of 3 and 4h groups were higher than those of 2 and 5h groups (P<0.05). The difference in cleavage, blastocyst and hatching blastocyst rates between 3 and 4h groups were not statistically significant (P>0.05). In conclusion, maturation time, concentration and timing of 6-DMAP treatment had effects on parthenogenetic blastocyst production of Cỏ goat oocytes, and Cỏ goat oocytes matured *in vitro* for 22 or 24h should be activated by 2mM 6-DMAP treatment for the 3 or 4h.

Keywords: *Cỏ goat oocytes, activation, 6-DMAP, parthenogenetic goat embryos.*

1. INTRODUCTION

The somatic cell nuclear transfer (SCNT) is an effective technique for producing transgenic or cloned animals. *In vitro* maturation and activation of oocytes are important steps for the success of the SCNT procedure. The parthenogenetic activation of oocytes is commonly used as a reference activation method during SCNT studies because the artificial activation of oocytes is a requirement of the SCNT procedure. Choosing the appropriate oocyte activation protocol can increase the chances of cloning success.

The parthenogenetic activation of oocytes performed by ionomycin, calcium ionophore (Kharche *et al*., 2016), ethanol (Pathak *et al*., 2013), strontium (Meo *et al*., 2004), cycloheximide, cytochalasine B, 6-dimethylaminopurine (6-DMAP) or electrical pulse (Kharche and Birade, 2013). Inactivation of M-phase-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) is a prerequisite used for release from metaphase arrest and formation of pronuclei (PN) in mammalian oocytes (Kishimoto, 2018). 6-dimethylaminopurine (a protein kinase inhibitor) has been shown to enhance oocyte activation through inactivation of MPF and MAPK (Valencia *et al*., 2023).

The maturation time of oocytes could be an important factor in the process of artificial activation (Shirazi *et al*. 2009). When using aged oocytes in an artificial activation process, MPK activity is reduced, therefore activation is more easily achieved. However, under the influence of artificial stimulation, the aged oocytes will have negative changes in the cytoplasmic and the components of the oocyte cytoskeleton, which affects embryo development (Dominko *et al*., 2000). The response of young oocytes to artificial

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stimulation is lower than that of aged oocytes, therefore, aged oocytes are often used as recipient oocytes in SCNT procedure.

Parthenogenetic activation of oocytes is significant for the cloned embryo production from somatic cell nuclear transfer in animals. An optimal activation protocol may enhance better or complete reprogramming of the reconstructed embryo and increase the blastocyst rate (Wang *et al.*, 2008). Cỏ goat is an indigenous goat breed in Vietnam, and they are an important genetic resource for the conservation of native Vietnamese goat biodiversity. However, until presently, there is no study on artificial activation of Cỏ goat oocytes in Vietnam which is very important for conducting any research on SCNT in future. This study evaluated the effect of maturation time, concentration and timing of 6-DMAP treatment on parthenogenetic blastocyst production of Cỏ goat oocytes.

2. MATERIAL AND METHODS

All chemicals were supplied by Sigma-Aldrich (St.Louis, MO, USA).

2.1. Collection of Cỏ goat ovaries and COCs

Cỏ goat ovaries were transported to the laboratory in Dullbeco phosphate buffer saline (DPBS) supplemented with antibiotics within 2hrs after collection from the slaughterhouse. Oocytes were collected 2-8mm diameter follicles on the ovarian surface using an aspiration method with a 5ml syringe containing Tyrode's Albumin Lactate Pyruvate-HEPES (TALP-HEPES) oocyte collection solution supplemented with serum and an 18G needle. After aspiration, the TALP-HEPES medium with harvested oocytes was transferred to a Petri dish and oocytes were searched by using a stereo microscope and evaluated according to the standards of Wani *et al*. (2000). After evaluation, cumulus-oocyte complexes were selected based on: (1) uniform cytoplasm and (2) the presence of at least three compact surrounding layers of cumulus cells.

2.2. In vitro maturation of goat oocytes

The COCs of Cỏ goat oocytes were washed three times in IVM medium either TCM 199 supplemented with 10% fetal calf serum (FCS), 50 ng/ml follicle-stimulating hormone (FSH), 10 ng/ml epidermal growth factor (EGF), 100μM cysteamine, 100 units/ml penicillin G potassium + 0.1mg/ml streptomycin sulphate. then transferred to 4-well plates containing 500μl of the *in vitro* maturation medium per well, and were then incubated under conditions of 38.5°C, 5% CO_2, in humidified air (50 oocytes per well).

2.3. Activation of oocytes

The activation procedures were performed using the method of Shirazi *et al*. (2009) with some modifications. Matured Cỏ goat oocytes were denuded of cumulus cells by vortex in the presence of 1mg/ml hyaluronidase in the TALP-HEPES medium. Then matured oocytes with the extruded first polar body (PB1) were selected under a stereo microscope. Matured oocytes with PB1 were treated with 5μM Ionomycin for 4min. Then, these oocytes were washed in TALP-HEPES supplemented with fetal bovine serum (FBS) and placed in Synthetic Oviductal Fluid (SOF) medium supplemented with 6-DMAP and 2.5% FBS, and were then incubated under conditions of 38.5°C, 5% CO_2 and 5% O_2 in humidified air.

2.4. Invitro culture

Following activation, presumptive zygotes were washed three times in SOF medium, transferred to the SOF medium supplemented with 2.5% FBS, and incubated at 38.5°C, 5% CO_2 and 5% O_2 in humidified air for 7 days. Cleavage was assessed 48h after activation and blastocyst and hatching blastocyst rates were recorded on days 6 and 7.

2.5. Evaluation of embryo cell number

Blastocysts were washed three times in the TALP-HEPES medium and then washed twice in PBS+0.3%PVP solution. Next, these blastocysts were transferred into a staining

solution (Hoechst 33342+Absolute Ethanol in a 1:9 ratio) and left overnight at 4°C. After incubation overnight in the staining solution, the blastocysts were washed in absolute Ethanol and then transferred to a Glycerol solution. Subsequently, the blastocysts were moved to a glass slide, each oocyte in a drop, and aligned along the length of the slide. A cover slip was placed over the slide, and the cell numbers were counted under a fluorescence microscope.

2.6. Statistical analysis

Data were expressed as Mean±SEM values and analysed by ANOVA, the tested differences between groups ($P<0.05$) was defined significantly.

3. RESULTS AND DISCUSSION

3.1. Influence of maturation duration

As shown in table 1, the cleaved, blastocyst and hatching rates of 22 and 24h groups were higher than those of 18 and 20h groups (60.48 and 60.35% vs 44.01 and 45.38%; 28.98 and 28.74% vs 9.98 and 12.06%; 16.315 and 16.02% vs 2.54 and 2.68%, respectively, $P<0.05$).

Table 1. Effects of maturation duration

Maturation duration	Σ	Cleaved (%)	Blastocyst (%)	Hatching blastocyst	Cells/ blastocyst
18h	82	36 44.01^{a}±2.42	8 9.98^{a}±2.19	2 2.54^{a}±1.99	139.78±2.79
20h	84	38 45.38^{a}±2.01	10 12.06^{a}±2.26	2 2.68^{a}±2.17	138.36±2.55
22h	88	53 60.48^{b}±2.27	25 28.98^{b}±2.16	14 16.31^{b}±2.51	139.72±2.82
24h	85	51 60.35^{b}±2.33	24 28.74^{b}±2.38	13 16.02^{b}±2.68	139.26±2.37

Note: The Mean in the same column with difference letter differ significantly (P<0.05).

The results in table 1 indicate that maturation time affects the production of parthenogenetic blastocyst of Cỏ goat oocytes and 22 or 24h of *in vitro* maturation time were the most suitable for activation of Cỏ goat oocytes. These results are similar to those reported by Kikuchi *et al.* (2000), Lan *et al*. (2005), and Shirazi *et al*. (2009), who also found that using aged oocytes for parthenogenetic blastocyst production resulted in higher blastocyst rate than young oocytes. These studies demonstrated the low response of young oocytes to parthenogenetic activation. Lan *et al*. (2005) also showed that 24h of *in vitro* maturation was suitable for the goat oocyte activation. However, our findings contrast with those reported by Shen *et al*. (2008). According to Shen *et al*. (2008), when bovine oocytes were artificially activated, there was no difference in the cleavage and blastocyst rates between 20 and 24h mature bovine oocytes. This indicates that parthenogenetic blastocyst production depends not only on maturation time but also on species.

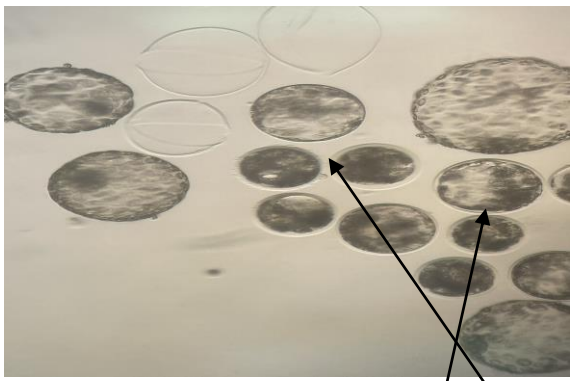

Figure 1. Parthenogenetic blastocyst and hatching blastocysts of Cỏ goat oocytes

The aged oocytes are sensitive to activating stimuli through decreased MPF activity (Kikuchi *et al.*, 2000), initiation of anaphase II, and partial exocytosis of theca granules (Shirazi *et al*., 2009). According to Kikuchi *et al*. (2000), aged oocytes show enhanced activation and higher fragmentation rates after parthenogenetic activation. The increased ability for parthenogenetic activation and fragmentation rate in porcine-aged oocytes may be due to the decline of MPF activity during prolonged culture.

3.2. Influence of concentration of 6-DMAP treatment on activation of Cỏ goat oocytes

Based on the results of *Experiment 1*, in *Experiment 2*, we used mature Cỏ goat oocytes with 22h of *in vitro* maturation to produce parthenogenetic blastocyst. The

results in table 2 indicate that the concentration (Conc) of 6-DMAP treatment affects the parthenogenetic blastocyst production of Cỏ goat oocytes. The cleaved, blastocyst and hatching rates of the 2mM group were higher than those of 1, 4 and 6mM groups (Table 2, P<0.05). This indicated that 2mM 6-DMAP treatment was the most suitable for Cỏ goat oocyte activation.

The results in table 2 are consistent with the reports of Mishra *et al.* (2007), Shirazi *et al.* (2009) and Kharche *et al.* (2015). According to Misha *et al.* (2007), Shirazi *et al.* (2009), and Kharche *et al.* (2015) the development of parthenogenetic embryos with 2-2.5mM concentration of 6-DMAP treatment was found effective among different species. Parthenogenetic activation of goat oocytes performed by ionomycin was followed by treatment with 6-DMAP (Haque *et al.*, 2011). The DNA status of reconstructed oocytes can be controlled by polar body extrusion or retention after activation. 6-DMAP enhanced the speed of pronuclear formation, suppressed polar body extrusion, inhibited second polar body extrusion and increased cell cycle progression of calcium-activated parthenogenetic embryos (Hyun *et al.*, 2021). Therefore, in this study, we used ionomycin and 6-DMAP to activate Cỏ goat oocytes.

Table 2. Effects of concentration 6-DMAP treatment

Conc	Σ	Cleaved (%)	Blastocyst (%)	Hatching blastocyst	Cells/ Blastocyst
1mM	84	38 45.81^{a}±2.67	16 19.54^{a}±2.86	4 4.98^{a}±2.41	138.31±2.85
2mM	89	66 74.65^{b}±2.09	38 42.96^{b}±2.01	26 29.62^{b}±2.71	139.06±3.01
4mM	88	53 60.48^{c}±2.27	25 28.98^{c}±2.16	14 16.31^{c}±2.51	139.72±2.82
6mM	81	36 44.95^{d}±2.82	14 17.99^{d}±2.56	12 15.09^{d}±3.12	139.55±2.47

3.3. Influence of timing of 6-DMAP treatment

Based on the results of *Experiment 2*, in *Experiment 3*, we used 2mM 6-DMAP to evaluate the effects of timing of 6-DMAP treatment for parthenogenetic blastocyst production of Cỏ goat oocytes. The results in table 3 indicate that the timing of 6-DMAP treatment affects the parthenogenetic blastocyst production of Cỏ goat oocytes. The cleaved, blastocyst and hatching rates of 3 and 4h groups were higher than those of 2 and 5h groups (Table 3, P<0.05). This indicated that 6-DMAP treatment for 3 or 4h was the most suitable for activation of Cỏ goat oocytes.

Table 3. Effect of timing of 6-DMAP treatment

Time	Σ	Cleaved (%)	Blastocyst (%)	Hatching blastocyst	Cells/ blastocysts
2h	85	43 50.91^{a}±1.96	18 21.82^{a}±2.63	8 9.92^{a}±2.73	139.12±2.87
3h	86	63 73.98^{b}±2.41	36 42.05^{b}±2.64	25 29.46^{b}±2.39	139.98±2.81
4h	89	66 74.65^{b}±2.09	38 42.96^{b}±2.01	26 29.62^{b}±2.71	139.06±3.01
5h	82	45 55.01^{a}±2.66	17 21.11^{a}±2.42	7 8.91^{a}±2.16	138.92±2.35

Our findings are consistent with reports of Choi *et al*. (2004) and Lan *et al.* (2005). According to Lan *et al.* (2005), goat oocytes should be activated by 6-DMAP for 3h. Choi *et al*. (2004) also showed that the timing of 6-DMAP treatment for 2h increased the rate of parthenogenetic blastocyst canine. The duration of exposure to 6-DMAP during activation affects DNA synthesis in the nucleus of parthenogenetic zygotes. Optimal timing 6-DMAP treatment can reduce the time oocytes spend in the G1 phase of the cell cycle, resulting in premature DNA synthesis. 6-DMAP is one of the most widely used activation protocols for parthenogenetic or cloned embryo production (Meo *et al.*, 2007). 6-DMAP is a serine protease inhibitor that can enhance pronucleus formation by blocking the activity of key cell cycle regulatory proteins such as MAPK (Park *et al*., 2010). 6-DMAP can suppress the MAPK activity faster and maintain the low level longer (Park *et al.*, 2010). MAPK activity dropped markedly after 1h and reached its lowest value 3h after electroporation (Nanassy *et al*. 2007). However, by 4h the activity increased again and remained at elevated levels until the timing of pronuclear

formation. That is why, the commonly used duration of treatment time with 6-DMAP for 3-4h. This finding is similar to the results of table 3 in this study.

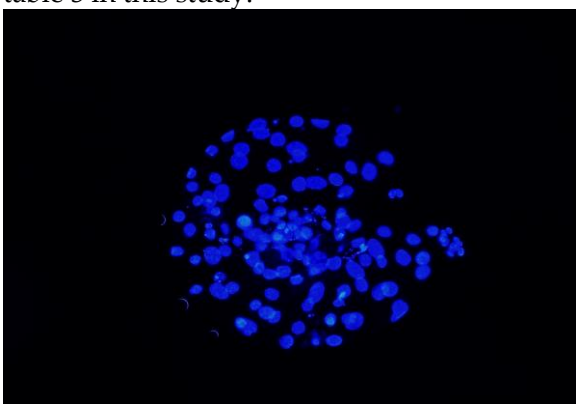

Figure 2. Parthenogenetic blastocyst of Cỏ goat oocytes

4. CONCLUSION

In conclusion, maturation time, concentration and timing of 6-DMAP treatment had effects on parthenogenetic blastocyst production of Cỏ goat oocytes, and Cỏ goat oocytes matured *in vitro* for 22 or 24h should be activated by 2mM 6-DMAP treatment for the 3 or 4h.

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THE EFFECT OF REPLACING ELEPHANT GRASS SILAGE WITH JACKFRUIT LEAVES SILAGE ON *IN VITRO* DIGESTIBILITY, RUMINAL FERMENTATION AND METHANE EMISSION

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ABSTRACT

The experiment aimed to evaluate the effect of replacing elephant grass (EG) silage with jackfruit leaves (JL) silage in the diet on *in vitro* nutrient digestibility, ruminal fermentation, and methane production using ruminal fluid from crossbred Saanen goats. The study was conducted using a completely randomized design, and treatments were developed by replacing EG silage with JL silage on a DM basis) at rates of 0, 25, 50, 75 and 100%, corresponding to JL0, JL25, JL50, JL75 and JL100. All diets were formulated to maintain a roughage-to-concentrate ratio of 60:40. The *in vitro* digestibility of DM, organic matter (OM), and crude protein (CP), as well as the concentration of NH_3-N, were not influenced (P>0.05) by the inclusion of JL silage in the diet. However, the digestibility of neutral detergent fiber (NDF) tended to increase (P=0.083; quadratic effect) in JL25 but gradually decreased as the replacement level increased to 100%. The total concentrations of volatile fatty acid (VFA) did not differ (P>0.05) among the treatments, although individual VFA concentrations altered (P<0.05) after 3 and 24h of incubation. During this period, the acetate increased linearly, and the propionate decreased linearly (P<0.01) after 24h of incubation. Total gas production, CH_4 and CO_2 volumes were not changed (P>0.05) by increasing the proportion of JL silage in the diet. The results of this experiment suggest that JL silage can completely replace for EG silage in goat diet containing 40% concentrate without negative impact on *in vitro* nutrient digestibility and ruminal fermentation.

Keywords: *Digestibility, elephant grass silage, jackfruit leaves silage, methane production, ruminal fermentation.*

1. INTRODUCTION

Goats are known for their efficiency in converting low-nutrient feed sources into high-nutrient products suitable for human consumption. They adapt well to various environmental conditions and require relatively low investment costs. Consequently, goat farming in Vietnam is rapidly expanding, both at the household and farm levels. Goats have a greater ability to consume a variety of feed sources compared to other ruminants (Nair *et al*., 2021). In recent years, Vietnam's economy has shifted its focus toward the industrial and service sectors. Investment in infrastructure for these sectors has significantly reduced the area available for grass cultivation. Consequently, finding alternative roughage sources to replace traditional grass has become a pressing issue. The utilization of agricultural by-products as feed for ruminants is encouraged to improve economic efficiency, contribute to environmental protection, and produce high-nutrient products for humans, such as meat and milk. Being a good method to prolong reserve time and maintain nutrient content while reducing labor resources, silage feeding has been applied in ruminant production in recent years.

Given its superior soil and cultivation conditions, the Mekong Delta has significantly expanded its fruit tree cultivation throughout the year, particularly in the area of jackfruit. Annually, jackfruit orchards are pruned 2-3 times, producing a large amount of fresh jackfruit leaves (JL), which represent a potential feed source for ruminants. JL are considered a preferred feed for goats (Van *et al*., 2005), containing 12.9% CP (Thanh *et al*., 2021). Additionally, JL were reported to contain alkaloids, flavonoids,

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tannins and steroids (Utari and Warly, 2021). Thanh *et al.* (2021) reported that JL contain up to 11.6% total tannin. Tannin inclusion in ruminant diet has high potential to reduce methane production (Thanh *et al*., 2022), methane emissions from ruminal fermentation are one of the primary sources of methane emissions in the agricultural sector. The improvement of ruminant diets is among the approaches listed in Vietnam's Nationally Determined Contribution at COP27, aimed at reducing greenhouse gas emissions.

Developing sustainable feeding strategies tailored to Vietnam's actual conditions is necessary to mitigate methane emissions while enhancing nutrient utilization and improving livestock productivity. Besides methane mitigation effect, the appropriate use of tanniferous plants, like JL, in ruminant diets can improve livestock productivity and slow the development of parasites *(Lileikis et al.*, 2023). However, few studies in Vietnam have been conducted to evaluate the potential of replacing grass silage with jackfruit leaves (JL) silage in goat diets. Thus, this study aimed to evaluate the effects of JL silage replacement for EG silage on *in vitro* ruminal fermentation patterns, digestibility, and CH_4 production using ruminal fluid from dairy goats.

2. MATERIALS AND METHODS

The study was performed at the Laboratory of Ruminant Production Techniques, Faculty of Animal Sciences, College of Agriculture, Can Tho University, Viet Nam. All procedures were performed according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

2.1. Experimental design and diets

Two *in vitro* experiments were performed in 1) 50ml bottles to determine nutrient digestibility and ruminal fermentation patterns and 2) 500ml bottles to determine CH_4 production. All experiments were carried out as a CR design. Treatment diets were developed by replacing JL silage for EG silage at 0, 25, 50, 75 and 100%, corresponding to JL0, JL25, JL50, JL75 and JL100 (DM basis). The diets, which contain a roughage to concentrate ratio of 60:40, were formulated to meet the nutrient requirements of lactating goats (NRC, 2007). Each experiment consists of 4 and 3 replicates which are separate sources of ruminal fluid collected from the crossbred Saanen goats, respectively.

Table 1. Feed ingredients and chemical composition

Item	JL0	JL25	JL50	JL75	JL100
Feed ingredients, % DM					
JL	0.00	15.0	30.0	45.0	60.0
Napier grass silage	60.0	45.0	30.0	15.0	0.00
Rice bran	16.9	16.9	16.9	16.9	16.9
Ground corn	5.40	5.40	5.40	5.40	5.40
Soybean meal	15.4	15.4	15.4	15.4	15.4
Premix	0.50	0.50	0.50	0.50	0.50
Limestone	0.90	0.90	0.90	0.90	0.90
DCP	0.60	0.60	0.60	0.60	0.60
Urea	0.30	0.30	0.30	0.30	0.30
Chemical composition, %DM (unless otherwise noted)					
DM	91.9	92.0	92.1	92.1	92.2
OM	87.9	87.7	87.6	87.4	87.2
Ash	12.1	12.3	12.4	12.6	12.8
CP	15.0	16.1	17.3	18.4	19.5
NDF	40.4	37.6	34.8	31.9	29.1
CF	23.5	21.1	18.7	16.4	14.0
EE	1.52	1.97	2.43	2.4	3.33
ME, MJ/kg DM	8.80	9.03	9.27	9.50	9.73

Note: OM: organic matter, Ash: total minerals, NDF: neutral detergent fiber, CF: crude fiber, EE: ether extract.

After harvesting, JL and EG were brought to the laboratory and chopped into small pieces of 0.5-1cm. Each kg of EG was ensiled with 30g of sugarcane molasses. Meanwhile, 1kg of JL was ensiled with 30g of sugarcane molasses, 2mg of Magniva Platinum 3 (7.5×10^{10}CFU/g *L. hilgardii*, 7.5×10^{10} CFU/g *L. buchneri* and 5.0×10^{10}CFU/g *P. pentosaceus* from Lallemand Animal Nutrition UK Ltd., UK), 750mg of ACTISAF

SC 47 STD containing 1.5×10^{10}CFU/g of *Saccharomyces cerevisiae* yeast cells (Phileo by Lesaffre, Marcq-en-Baroeul, France), and 5g of NaCl. After 4w, the quality of the ensiled samples was assessed, and the bag with the best quality with a pH in the range of 3.8-4.2 was selected for *in vitro* experiments.

2.2. Inoculum and medium solution

Ruminal fluid was collected from 4 crossbred Saanen goats. Goats were fed the basal diet consisting of EG and an 18% CP concentrate (roughage:concentrate 60:40, wt/wt on DM basis) twice a day at 6:00 and 17:00 for 1wk prior to sampling. Approximately 200ml of ruminal fluid was collected from each goat 2h after the morning feeding using a stomach tube passed through the mouth. Ruminal fluid was filtered through a metal sieve with a pore size of 1mm under a stream of CO_2 at 39°C. Donor fluid from each goat was kept separately and served as statistical replicates during *in vitro* experiments. Ruminal inocula were diluted (1:4, vol/vol) in the medium solution which was prepared according to Menke and Steingass (1988).

2.3. *In vitro* incubation

In experiment 1, 0.3125g DM of substrates was weighed into each 50ml bottle. Thereafter, 25ml of the mixture comprised of ruminal fluid: medium solution (1:4 vol/vol) was added. Bottles were incubated in a shaking incubator (ISS-4075R, Jeiotech, Korea) at 120rpm, 39°C for 72h. At 24h, the fermentation reaction was stopped by placing bottles in the ice, and the pH value of the fermentation solution was measured immediately (HI-5522, Hanna Instruments Inc., USA). The fermented liquor was then filtered through four layers of cheesecloth and acidified with H_2SO_4 1M (9:1, vol/vol), centrifuged at 10,000×*g* for 15min. The supernatant was obtained and stored at -35°C for further analysis of NH_3-N and VFA. After 72h of incubation, the *in vitro* digestibility of DM (DMD), CP (CPD) and NDF (NDFD) was determined according to Van Soest and Robertson (1985).

In experiment 2, 6.25g DM of substrates were weighed into the 500ml glass bottles, then 400ml of a mixture consisting of ruminal fluid and medium solution at a 4:1 ratio was added. Bottles were connected to multi-layer foil gas sampling bags (22952, Restek, USA) and incubated in a shaking incubator (ISF-7200R, Jeiotech, Korea) at 120rpm, 39°C for 72h. Total gas production was measured at 24, 48 and 72h of incubation. At each time point of collection, the gas production was recorded and transferred into another respective gas sampling bag and stored until the determination of CH_4 concentration.

2.4. Chemical analysis and calculation

Feed samples were analyzed for DM, OM, CP, CF and EE following the methods outlined by AOAC (1990); NDF was analyzed according to the method of Van Soest *et al*. (1991); chemical composition was calculated and presented on a DM basis; NH_3-N content in ruminal fluid was analyzed using the Kjeldahl method (AOAC, 1990), individual VFA concentrations were determined by gas chromatography (Trace 13010, Thermo Scientific, USA) and concentrations of produced gases were measured using a gas analyzer (GeoTech GA5000, Queensway, UK).

The digestibilities of DM, OM, CP, and NDF were calculated using the formula 100×(nutrient content of the substrates before incubation–nutrient content of the substrate after incubation)/nutrient content of the substrates before incubation.

2.5. Statistical analysis

Data were statistically analyzed with the GLM procedure for CRD using SAS 2021). The statistical model was $Y_{ij} = \mu + D_i + \varepsilon_{ij}$, *where* Y_{ij} *= the dependent variable,* μ *= the overall mean,* D_i *= the diet effect, and* ε_{ij} *= the random residual error*. Significant differences among diet means were statistically compared using Tukey test. Significant effect of diet was declared at $P<0.05$.

3. RESULTS AND RESULTS

3.1. Nutrient digestibility

The replacement of JL silage for EG silage in this experiment did not affect (P>0.05; Table 2) the digestibility of DM, OM, and CP after 72h incubation. The DM digestibility ranged 51.0-61.5%, OM digestibility ranged 54.5-62.9%, and CP digestibility ranged 24.5-34.9%. The NDF digestibility tended (P=0.083) to increase (quadratic effect) when 25% of EG silage was replaced by JL silage, but then gradually decreased from JL25 to JL100. These results differ from previous experiments which report that using tanniferous plants in ruminant diets could reduce nutrient digestibility (Gemeda and Hassen, 2015; Terranova *et al*., 2018; Vargas-Ortiz *et al*., 2022). The discrepancy could imply the role of *Saccharomyces cerevisiae* in the ensiled process in improving nutrient digestibility in the current study. Besides, the positive effect of *S. cerevisiae* on rumen microbial development and animal performance in ruminants has been noticed before (Sales, 2011; Wang *et al*., 2022; Dai *et al*., 2023).

Table 2. Nutrient digestibility after 72h incubation

Item	Treatment					SEM	P	Contrast	
	JL0	JL25	JL50	JL75	JL100			L	Q
DMD, %	61.5	60.4	60.0	51.0	51.7	3.62	0.145	0.279	0.587
OMD, %	62.7	62.6	62.0	62.9	54.5	3.45	0.395	0.477	0.477
CPD, %	24.5	31.8	31.6	30.1	34.9	1.91	0.363	0.482	0.137
NDFD, %	53.2	62.2	60.5	57.7	56.9	2.87	0.263	0.184	0.083

Note: Linear (L) and Quadratic (Q) effect of treatment

3.2. Ruminal fermentation patterns

At 0h, replacing JL silage for EG silage in the diet significantly altered the pH (P<0.05, Table 3). However, there were no significant differences in other ruminal fermentation parameters among the treatments (P>0.05). NH_3-N concentration ranged from 31.4 to 48.0 mg/dl, and total VFA ranged from 19.5 to 23.3mM. Additionally, the proportions of individual VFAs did not differ among treatments.

After 3h of incubation, VFA was noteworthy higher than at 0h, suggesting that fiber in EG silage and JL silage are easy-fermentable sources. No effects of JL silage on the values of pH, NH_3-N, or total VFA were observed (P>0.05), although changes in the composition of individual VFAs were noted. Acetate linearly increased (P<0.05) as the level of JL silage in the diet increased from 0 to 75% (70.2-76.2%) while the proportion of propionate was not affected (P>0.05) by the level of JL silage in the diet. Consequently, the acetate:propionate ratio tended to increase linearly (P=0.069) from JL0 to JL75. The proportions of iso-butyrate, butyrate, iso-valerate, and valerate linearly decreased (P<0.05) with increasing levels of JL silage. These reductions indicated that protein degradability was limited in tannin-containing treatments (Costa *et al*., 2018; Roca-Fernández *et al*., 2020), despite the lack of discrepancy in NH_3-N concentration.

At 24h incubation, ruminal pH ranged 6.75-7.04, and NH_3-N ranged 57.7-94.6 mg/dl (P>0.05). The total VFA concentration was not different (P>0.05) among treatments, ranging 137-169mM. Acetate proportion at 24h was highest (P<0.05) in the JL100 treatment (73.2mM) compared with those in JL25 and JL50 (67.3 and 67.2mM). Meanwhile, propionate levels gradually decreased (P<0.05) from 24.2% in JL0 to 19.6% in JL100. As a result, the acetate to propionate ratio increased (P<0.01) from 2.83 in JL0 to 3.76 in JL100. Valerate accounted for 1.42% in JL75, which was 0.35% lower (P<0.05) than those in JL50 and JL75. The increase in acetate levels in treatments containing JL silage may be attributed to the enhancement of acetogenesis (Tan *et al*., 2011). The observed decrease in propionate levels in this experiment aligns with the findings of Guerreiro *et al.* (2021), which reported a similar trend when increasing levels (0-10% DM) of *Cistus ladanifer* L. extract, which is high in condensed tannin, in the diet.

Table 3. Ruminal fermentation patterns after 24h incubation

Time	Variable	Treatment					SEM	P	Contrast	
		JL0	JL25	JL50	JL75	JL100			L	Q
0h	pH	8.00^{ab}	7.58^{b}	7.79^{ab}	8.25^{ab}	8.42^{a}	0.17	0.020	0.789	0.092
	NH_3-N, mg/dL	31.4	39.3	48.0	39.6	36.8	7.42	0.630	0.257	0.448
	Total VFA, mM	23.3	23.3	21.0	19.9	19.5	2.00	0.540	0.420	0.922
	Acetate, %	80.1	81.5	81.0	80.4	81.9	1.76	0.940	0.975	0.457
	Propionate, %	14.2	13.4	13.3	13.6	12.2	0.98	0.713	0.999	0.385
	Iso-butyrate, %	0.69	0.67	0.79	0.78	0.84	0.11	0.778	0.694	0.940
	Butyrate, %	4.17	3.54	3.83	4.14	3.91	0.93	0.989	0.964	0.614
	Iso-valerate, %	0.38	0.38	0.43	0.43	0.48	0.08	0.903	0.854	0.914
	Valerate, %	0.53	0.50	0.62	0.60	0.62	0.06	0.591	0.561	0.903
	Acetate:Propionate	5.82	6.26	6.12	6.01	6.86	0.57	0.751	0.843	0.406
3h	pH	7.19	7.27	7.04	7.32	7.18	0.10	0.400	0.718	0.524
	NH_3-N, mg/dL	49.3	56.2	50.0	45.7	49.8	9.08	0.948	0.811	0.522
	Total VFA, mM	71.3	64.5	60.0	64.5	57.8	6.77	0.678	0.607	0.344
	Acetate, %	70.2	72.3	72.3	76.2	72.2	1.53	0.148	0.026	0.523
	Propionate, %	22.7	23.0	23.2	21.3	25.4	1.10	0.186	0.176	0.134
	Iso-butyrate, %	0.56^{a}	0.47^{ab}	0.43^{ab}	0.26^{b}	0.25^{b}	0.06	0.005	0.026	0.888
	Butyrate, %	5.29^{a}	3.27^{ab}	3.23^{ab}	1.67^{b}	1.61^{b}	0.72	0.016	0.027	0.345
	Iso-valerate, %	0.51^{a}	0.39^{ab}	0.36^{ab}	0.22^{b}	0.20^{b}	0.06	0.017	0.052	0.657
	Valerate, %	0.71^{a}	0.58^{ab}	0.51^{ab}	0.35^{b}	0.32^{b}	0.07	0.005	0.026	0.634
	Acetate:Propionate	3.09	3.21	3.16	3.60	2.84	0.21	0.212	0.069	0.238
24h	pH	6.78	6.91	6.75	7.04	6.88	0.10	0.327	0.250	0.632
	NH_3-N, mg/dL	57.7	77.8	94.6	81.8	74.3	14.0	0.486	0.143	0.396
	Total VFA, mM	137	142	158	169	164	11.2	0.245	0.133	0.874
	Acetate, %	67.8^{ab}	67.3^{b}	67.2^{b}	72.0^{ab}	73.2^{a}	1.45	0.021	0.004	0.139
	Propionate, %	24.2^{a}	23.0^{ab}	21.7^{ab}	20.0^{ab}	19.6^{b}	1.01	0.027	0.002	0.785
	Iso-butyrate, %	0.67	0.74	0.70	0.65	0.65	0.04	0.534	0.377	0.345
	Butyrate, %	5.11	6.45	7.84	5.13	4.25	1.36	0.403	0.489	0.113
	Iso-valerate, %	0.71	0.78	0.77	0.78	0.71	0.07	0.894	0.983	0.355
	Valerate, %	1.52^{ab}	1.77^{a}	1.77^{a}	1.42^{b}	1.54^{ab}	0.08	0.024	0.224	0.072
	Acetate:Propionate	2.83^{b}	2.95^{b}	3.12^{ab}	3.60^{ab}	3.76^{a}	0.20	0.007	<0.001	0.556

3.3. Gas production after 72h incubation

Table 4. Total gas and methane production after 72h incubation

Item	Treatment					SEM	P	Contrast	
	JL0	JL25	JL50	JL75	JL100			L	Q
Total gas, ml	1,496	1,312	1,442	1,421	1,559	111	0,619	0,467	0,624
CH_4, %	12.2	12.8	12.6	12.7	13.5	0.74	0.760	0.983	0.481
CH_4, ml	184	166	183	181	184	21.3	0.677	0.568	0.810
CH_4, ml/g DM	29.5	26.6	29.3	29.0	33.8	3.40	0.677	0.612	0.962
CO_2,%	67.6	58.3	70.9	60.9	65.6	4.35	0.295	0.463	0.548
CO_2, ml	1.012	766	1.023	886	1.011	101	0.333	0.612	0.962
CO_2, ml/g DM	162	123	164	142	162	16.1	0.333	0.463	0.548

The use of JL silage as a replacement for EG silage in the diet did not significantly affect (P>0.05) total gas production or the proportions of CH_4 and CO_2 under *in vitro* conditions. The total gas volume ranged from 1,312 to 1,559ml after 72h of incubation, with CH_4 proportions ranging 12.2-13.5% and CO_2 proportions ranging 58.3-70.9%. Consequently, the volume of CH_4 produced after 72h of incubation was between 166 and 184ml, with no significant differences (P>0.05) among treatments. This study was contrary to the previous research reported by Rira *et al.* (2015), in which total gas and CH_4 production gradually decreased with the tannin-rich plant's proportion from *Gliricidia*

sepium, Leucaena leucocephala, and *Manihot esculenta* at 0, 25, 50, 75 and 100% in sheep diet. The variations in total gas and methane production can be attributed to differences in substrate composition, doses, and the reaction mechanisms of different sources of tannins and animal species used in various experiments.

4. CONCLUSION

Combined results suggest that JL silage can totally replace EG silage in goat diet containing 40% concentrate without negative impact on nutrient digestibility and ruminal fermentation.

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PALATABILITY OF PET FOOD AND SOME EVALUATION METHODS

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ABSTRACT

The pet food industry is experiencing a globally strong growth. Quality control and the development of pet foods primarily rely on palatability assessment trials. Palatability is a crucial factor determining the amount of food that pets consume, reflecting their preferences and acceptance levels. Measuring palatability not only helps evaluate the degree of preference by pets but also provides data to improve product quality. Currently, traditional methods for assessing palatability, such as single-bowl and two-bowl tests, are widely used in pet research. The single-bowl test offers only one type of food and evaluates consumption amount, while the two-bowl test allows pets to choose between two types of food, thereby determining which one they prefer. Both methods have certain merits in analyzing pet feeding behaviors, but also remains some limitations. To enhance palatability assessment, modern studies are developing more complex methods, such as measuring physiological responses (e.g., salivation, heart rate), observation of detailed feeding behaviors, and chemical analysis of food components. This article clarifies these methods for measuring pet food palatability.

Keywords: *Acceptance, palatability, pet food, dogs, cats, preference.*

1. INTRODUCTION

The pet food industry is an important segment of the animal care and is rapidly expanding globally. In Vietnam, although this market remains small, there has been intense competition between domestic companies and multinational corporations for market share. In 2020, pet food revenue in Vietnam reached USD 53.9 million and is projected to increase to USD 105.7 million by 2028 (https://growthmarketreports.com). This growth is largely due to the increasing demand for specialized nutritional products for pets.

Globally, pet food sales have soared from USD 78.1 billion in 2011 to USD 114.8 billion in 2021 and USD 125.1 billion in 2023. In the animal feed industry, dog and cat food accounted for 96% of total revenue in 2021, equivalent to USD 110.6 billion. The large market share of dog and cat food may be attributed to them being the most commonly kept pets in households. It is projected that by 2026, global pet food revenue will reach USD 156.9 billion, with dog and cat food contributing approximately USD 152 billion (Euromonitor International, 2022; https://www.imarcgroup.com/pet-food-market).

The rate of pet ownership in developed countries has also increased significantly in recent years. This has led to the introduction and improvement of numerous new products to meet the diverse needs of pet owners (Aldrich and Koppel, 2015). The pet food industry has applied a variety of pet models to evaluate feed quality. These models provide rapid results at reasonable costs. The main criteria are usually based on feeding habits and compliance with the pet's nutritional standards (according to the Association of American Feed Control Officials-AAFCO), digestibility, fecal consistency and quality, and particularly palatability (Companion Animals New Zealand, 2020).

Palatability has become a core criterion in product development, influencing everything from ingredient selection and processing methods to quality control. Palatability is a combination of the physical

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and chemical properties of the feed that can stimulate or inhibit the pet's feeding behavior during the pre-absorptive phase (National Research Council, 2006; Aldrich and Koppel, 2015). Unlike humans, pets cannot clearly express their preferences; therefore, their feeding behavior is the only indicator. The concept of "liking" becomes important when humans attempt to feed pets industrial diets instead of their original natural prey. Therefore, developing scientific methods to assess palatability and pet preferences is extremely necessary in developing appropriate pet foods.

2. PALATABILITY AND PREFERENCES OF PETS

As the number of commercial pet food products continues to increase, palatability has become a paramount criterion for evaluating product effectiveness. Palatability is not related to nutritional requirements but reflects the perception and preferences of pets during feeding (Stasiak, 2002). A feed is considered palatable when pets readily accept and consume it without coercion (Stasiak, 2002; Tobie *et al.,* 2015; Aldrich and Koppel, 2015). However, the results of palatability assessment trials can be influenced by various factors such as the type of test animals, their feeding behaviors, and the characteristics of the feed (including flavor, texture, and temperature). Therefore, controlling these factors is crucial to ensure accurate and reliable palatability evaluation results.

3. ANIMAL FACTORS AFFECTING PALATABILITY

Selecting appropriate tested animals for evaluating feed palatability is crucial to ensure the accuracy and scientific validity of the results obtained. Each animal species has different biological characteristics, nutritional requirements, behaviors, eating habits, and preferences. Dogs (*Canis lupus familiaris*) and cats (*Felis catus*) are currently the two most popular pets, and there are significant differences between them-not only in behavior but also in how they perceive and consume food.

Dogs can consume a wide variety of foods, including insects, grass, feces, and even carrion. They also have the ability to chew bones and eat animal parts without difficulty. Dogs have a special oral structure with large canines and small molars, allowing them to tear and grind food quickly. In the mouths of dogs, saliva is produced from four major salivary glands but does not contain amylase, an enzyme responsible for starch breakdown in humans (Bosch *et al.*, 2015). This means that the digestion of food in dogs primarily occurs in the stomach and small intestine, not starting in the mouth. Dogs often exhibit rapid eating behavior, gulping down food without thorough chewing. After eating, dogs may regurgitate ingested food and eat it again-a behavior stemming from an instinct to protect food from competition with other animals.

Cats are solitary predators and often lie in and wait for opportunities to kill small prey such as birds and mice. When they catch prey, cats usually eat immediately, particularly favoring food that is still warm, with a temperature close to the body temperature of their prey. If they have to eat larger prey, cats will tear off small portions to eat gradually. Cats rarely eat cold carcasses because they prefer fresh food; this is instinctive behavior to ensure optimal nutrient intake and avoid risks from harmful bacteria (Becques *et al.,* 2014; Eyre *et al.,* 2022). The large canines of cats help them bite and kill prey, while small incisors and knife-like molars assist in tearing flesh. Like dogs, cat saliva also does not contain amylase, and the digestion of starch does not begin in the mouth (Eyre *et al.,* 2022; Jobin *et al.,* 2000). However, cats have a highly developed olfactory system, helping them easily detect food odors and slight changes in food composition or flavor, and if they dislike it, they will refuse to eat. This makes cats more finicky eaters compared to dogs. Cats are

highly selective when eating, often sniffing thoroughly before deciding whether to eat, paying special attention to the freshness and safety of the food.

When assessing cats' reactions to food, facial expressions—including movements of the face, tongue, eyes, and nose-should be the first behaviors evaluated. When cats like the food, they tend to lick and sniff the bowl, lick their lips, and groom their face. Conversely, when they dislike the food, cats still exhibit licking and sniffing behaviors but also include nose-licking. However, the subtle differences between lip-licking and nose-licking make it challenging to distinguish these behaviors during palatability assessment. The time cats spend sniffing the food can be a measure of palatability (Van den Bos *et al.,* 2000).

Cats tend to eat multiple small meals throughout the day, rather than consuming large amounts in one sitting like dogs. Both dogs and cats like new food, but their reactions to changes in food can differ by individual and age. Puppies often tend to explore and more readily accept new types of food compared to adult dogs, as they are in a stage of learning and interacting with their environment (Bourgeois *et al.,* 2006; Péron and Tobie, 2018). Dogs more readily accept various types of food than cats, partly due to their diverse eating habits and survival instincts. Conversely, cats exhibit higher selectivity when exposed to food. This makes developing cat foods more complex and requires higher standards of quality and consistency.

Several factors need to be controlled when conducting palatability trials, such as individual animals, hunger levels, and climate, to ensure accurate results. Cats may exhibit side preferences when they only like to eat from a bowl placed on one side (left or right) regardless of the type of food. This leads to skewed trial results; therefore, individual animals need to be carefully screened before testing. Additionally, the satiety or hunger level of animals before testing can affect the amount of food consumed. To avoid this, test animals are often fasted before the trial. Climate can also impact the feeding behavior of pets. Cats often eat less during winter, so they need time to adapt before starting the formal trial (Tobie *et al.,* 2015; Péron and Tobie, 2018).

4. PALATABILITY ENHANCERS

Cats are obligate carnivores with high requirements for nutrients such as vitamin A, arachidonic acid, taurine, and niacin (Zaghini and Biagi, 2005). Without adequate animal-derived protein, cats can suffer severe nutritional deficiencies. This explains why they often appear finicky and reluctant to consume processed foods. Their behavior essentially reflects their biological needs rather than mere pickiness. The meticulous selection of food by cats can also be considered an indicator for assessing a product's palatability.

Flavoring agents or palatability enhancers in pet food include proteins, amino acids, carbohydrates, fatty acids, vitamins, and minerals. The purpose of these components is to stimulate taste receptors, particularly the umami receptors T1R1 and T1R3. Cats have a strong affinity for umami-tasting compounds (Salaun *et al.,* 2016; Alegría-Morán *et al.,* 2019). Umami, meaning "delicious" in Japanese, is one of the five basic tastes (sweet, sour, salty, bitter, and umami) that humans and animals can perceive.

In the pet food industry, hydrolyzed animal proteins have been used to create palatability enhancers through the Maillard reaction, which helps to add more appealing flavors to the food. Additionally, factors such as animal proteins, essential amino acids, and minced or pureed meats containing fats are very important flavor components for cats. Flavoring agents can be in dry or liquid form and are often added to dry foods after extrusion to improve taste. Cats tend to prefer wet or canned foods (because the

moisture content of canned foods is very close to that of meat: 70-85%) over dry foods, and since the processing techniques are more complex, less flavoring agents are needed to ensure palatability (Zaghini and Biagi, 2005; Pekel *et al.*, 2020).

5. ASSESSMENT OF PET PALATABILITY

Taste is the sensation produced when taste buds in the oral cavity are stimulated by chemical receptors. Odor consists of volatile components in food that stimulate olfactory receptors in the nasal cavity. During feeding, taste combines with olfaction to allow animals to perceive flavors through receptors in the mouth, nose, and larynx. Palatability begins when the pet comes into contact with food and depends on flavor, shape, temperature, size, texture, homogeneity of the food, and possibly previous feeding experiences (Alexander *et al.,* 2020). Palatability can be influenced by other factors such as the animal itself, time, previous experiences, humans, and the environment.

There are two types of pet palatability tests: consumption and non-consumption trials (Peachey and Harper, 2002). In non-consumption tests, reactions can be natural or conditioned (e.g., conditioned reflexes of dogs toward food), or tests based on conditioned actions (e.g., Skinner box experiments where animals learn to associate actions with rewards or avoidance of rewards). The most common methods to measure palatability are the single-bowl test (to measure acceptance level) and the two-bowl test (to assess preference) (Bradshaw *et al.,* 1996; Driscoll *et al.,* 2009). The single-bowl test measures the amount of food a pet consumes without relating to flavor. The two-bowl test evaluates the animal's preference for one type of food over another.

The aroma of food is a combination of olfactory signals and can be influenced by factors such as humidity and temperature. Both dogs and cats have the Jacobson's organ (vomeronasal organ), which helps amplify food odors and plays an important role in recognizing and responding to food. Odor is the initial evaluative behavior of pets when they first approach and sniff the food. Feeding preferences of individuals within the same group can vary. Some animals reject a certain type of food while others prefer it. This may be the result of conditioned aversions formed after previous exposure to unpleasant food. Additionally, some animals may exhibit neophobia (fear of new things) or neophilia (preference for new things) through their reactions to unfamiliar food.

5.1. Single-bowl test method

In this method, the test pets are provided with only a single type of food. This method determines the daily food intake of pets by comparing the mass of food offered with the amount of leftover food after a certain period of time. The trial typically involves 8-10 pets and is repeated over 5 days or more to minimize the impact of environmental factors on the results. The advantages of this method are low cost, no need for animal training, and easy application for both kenneled animals and household pets (Aldrich and Koppel, 2015). The disadvantage is that it only assesses the level of acceptance of the animals toward the food, without providing information on preference or liking; kenneled animals may react differently to food compared to house pets (Péron and Tobie, 2018).

To overcome this drawback, it is necessary to adjust the diet to acclimate the test animals over approximately 4-5 days before starting the trial, to ensure uniformity. However, this process is quite time-consuming and is often not applied in at-home trials. In addition, the amount of food provided to the animals should not exceed their normal caloric intake (plus 10%) to avoid overweight conditions, which could affect the test results. This method needs to be improved by monitoring additional indicators such as heart rate, pupil dilation, respiratory rate, activity level, body

movements, eating speed, and facial expressions related to the acceptance or rejection of the food to provide a more comprehensive evaluation.

5.2. Two-bowl test method

This palatability assessment method is widely used and provides the most reliable results in research on dog and cat nutrition. In this method, two types of food are offered simultaneously to the pets for a specified period, allowing them to choose (Tobie *et al.*, 2015). This approach permits pets to sniff and select before eating, providing a more comprehensive evaluation of their preferences. To avoid bias due to bowl placement, the trial needs to be conducted twice with the positions of the food bowls swapped between tests. The animal's initial sniffing and biting behaviors often reflect the attractiveness of the food's aroma. The test typically lasts 15-30min or until one of the two bowls of food is completely consumed. Animals are fed in the morning after an overnight fast, and the amount of food in each bowl must ensure sufficient daily caloric intake. The preferred food is determined by comparing the total amount of food consumed from each bowl (Aldrich and Koppel, 2015).

The two-bowl test can be applied to both kenneled animals and household pets, but in-home testing may reduce accuracy due to difficulties in controlling external factors. During the trial, animals are usually placed in individual testing chambers to avoid competition or environmental influences. Repeating the test and changing the positions of the bowls help ensure the accuracy of the results.

Dogs often tend to eat from both bowls, so careful monitoring of which bowl is approached first, which is finished first, and their feeding behaviors is necessary. The number of animals participating in the two-bowl test typically involves 10 animals over 5-6 days or 20 animals over 2-4 days to collect approximately 50-60 observations (Aldrich and Koppel, 2015). For cats, using 8 trained cats tested for 2h each day over 5 days can also yield accurate results.

An advantage of the two-bowl test is its ability to evaluate new flavors and compare test diets with control diets. This allows businesses to easily improve or develop new types of pet food. A disadvantage of this method is that if evaluating multiple products, the test must be repeated many times, making it time-consuming.

5.3. Other method to determine palatability

The lever-pressing test method involves training animals to rotate or press a lever with their nose to select the type of feed they prefer to consume more (Morris and Rogers, 1978). This method can yield results different from the two-bowl test and requires a more complex testing apparatus. It demands training time and animals with higher cognitive abilities but requires fewer subjects. However, this method does not evaluate the impact of experimental conditions and is only effective for feed types with clear distinctions. Currently, palatability tests are mainly operator-controlled and are not standard methods for determining the dietary preferences of dogs or cats.

6. CONCLUSION

Palatability in dogs and cats is a critical measure that reflects the amount of food consumed and the preference level for one type of food over another. Researchers often use single-bowl or two-bowl testing methods to assess palatability; however, these methods have several limitations. This indicates the need for continued research and development of palatability assessment methods to more accurately evaluate food preference in dogs and cats.

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