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EFFECTS OF SUPPLEMENTING CULTURED *Cordyceps militaris* MUSHROOM MYCELIA IN THE PREGNANT SOW'S DIET ON THE HEALTH AND PERFORMANCE OF THE MOTHERS AND THEIR SUCKLING PIGLETS

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ABSTRACT: Present study aimed to evaluate the effects of supplementing cultured Cordyceps mushroom mycelia (CMM) in the diets of pregnant sows on the productivity of the mothers and their suckling piglets during their first week of age. A total of 30 pregnant F1 (Landrace x Yorkshire) sows were randomly allocated to 5 dietary treatments with 6 replicates each: Control (sows fed the basal diet), and T30, T50, T100, and T200, where sows were fed the basal diet supplemented with 30, 50, 100, and 200 g of dried CMM, respectively. The animals were individually housed and fed twice daily. The performance and health status of the sows and their piglets were recorded accordingly. The results showed that the inclusion of CMM in the diets of pregnant and lactating sows affected the performance and health status of both the mothers and their piglets. For the piglets, the total number of piglets born and alive was higher in the T50, T100, and T200 groups compared to the control and T30 groups, but there was no effect on the survival rate at 7 days old. Daily gains per piglet were higher in the T30, T50, and T100 groups compared to T200 ($P<0.05$). For the sows, daily feed intake was lower in the T30 group compared to the other treatments ($P<0.05$). The values of gross energy in the milk produced by the sows were higher in the control, T30, T50, and T100 groups compared to T200 ($P<0.05$). Both the piglets and the sows on diets supplemented with CMM experienced fewer health problems than those on the control diet ($P<0.05$). In conclusion, the supplementation of 50 and 100 g of CMM per day in the diets of pregnant and lactating sows improved litter size and health status but did not affect the performance of either the mothers or their piglets.

Keywords: Cordyceps mushroom mycelia, Health status, Pregnant sows, Suckling piglets, Weight gain.

INTRODUCTION

In Vietnam, "Dong Trung Ha Thao" in Vietnamese and "Winter Worm Summer Grass" in English have been used as traditional folk medicine for hundreds of years (Thanh et al., 2018; Lu, 2023). Cordyceps is a phenomenon in which worms of the *Hepialus* genus in the *Lepidoptera* family are parasitized by a fungus with the scientific name *Cordyceps sinensis* (Berk.) of the *Ascomycetes* family (Kobayashi, 1982). Cordyceps belongs to the fungus family and is parasitic on the bodies of insects (Kumar et al., 2015). Under natural conditions in the winter, the fungi parasitize insects, develop into fungal mycelium (the asexual stage), use nutrients from the insect's body, and kill the insect. In the summer, the asexual fungal mycelium changes to the sexual stage, forming a mushroom—a structure that contains sexual spores and emerges from the ground, although the root is still attached to the stem (Figure 1A). Cordyceps has long been used in traditional Chinese medicine with the belief that it can treat various diseases. Under artificial conditions, *Cordyceps militaris* easily formed fruiting bodies in the culture environment (Figure 1B).

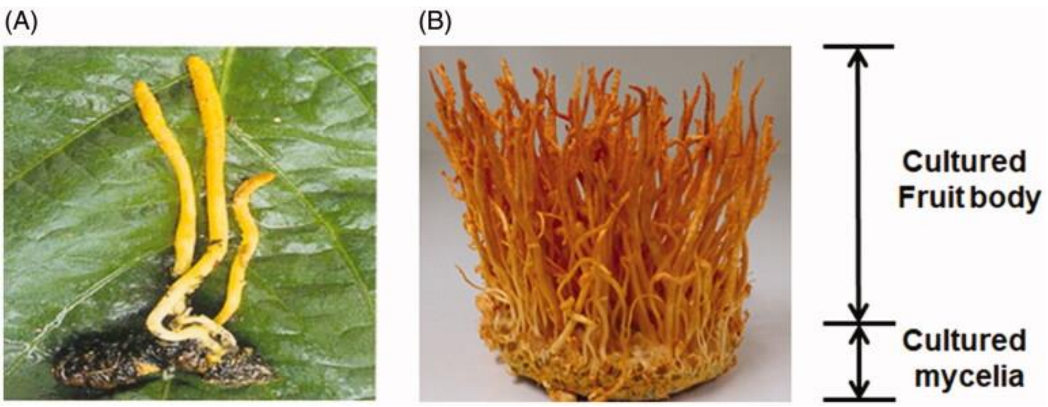

Figure 1 - Natural (A) and cultured (B) *Cordyceps militariris* (Adapted from Chou et al., 2024)

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The fruiting body of *Cordyceps* is collected from infected pupae or larvae, while the mycelia or corpus is considered a by-product, referred to as "Cordyceps mushroom mycelia – CMM (Sharma et al., 2024) ". It is believed that the fruiting body and the mycelium or corpus of *C. militaris* have different functions due to the former growing above ground and the latter existing underground (Hong et al., 2007). According to Hur (2008), the *Cordyceps* corpus contains most of the essential amino acids and essential fatty acids, such as linoleic acid (n-6) and alpha-linolenic acid (n-3), as well as biologically active compounds like adenosine and cordycepin. Additionally, Boontiam et al. (2020a,b) and Omthonglang et al. (2021) described the *Cordyceps* spent mushroom substrate (CMM) as including the mushroom corpus and the substrates from the culture medium of *Cordyceps* mushrooms. In this study, the CMM was used, and its bioactive compounds, as well as the concentrations of amino acids and fatty acids, were analyzed.

Koh et al. (2003) found that *Cordyceps* mycelium can serve as an alternative antibiotic growth promoter to improve weight gain and immunity in broiler chickens. Additionally, the inclusion of 1 g/kg of fermented *C. militaris* significantly increased weight gain in broiler chickens (Han et al., 2015). In weaning pigs, diets supplemented with 1,000 µg/kg fermented *Cordyceps* promoted growth performance and cell-mediated immunity (Cheng et al., 2016). In growing pigs, supplementation with CMM at 2 g/kg of diet increased growth performance, immunoglobulin secretion, and antioxidant capacity, while lowering leukocyte percentage, cholesterol, and MDA concentrations (Boontiam et al., 2020a). Ahtwichai et al. (2019) supplemented 2.45-4.9 mg cordycepin in diets for pregnant sows until weaning and reported that *C. militaris* supplementation affected reproductive performance, altered oxidative status, and reduced the fecal score in suckling piglets. Therefore, supplementing feed with *Cordyceps* substrates might provide an alternative approach in livestock production, improving both animal health and performance.

To our knowledge, there are few or no published reports on using *Cordyceps militaris* mushroom mycelia as a feed additive for sows and their suckling piglets. We hypothesized that the presence of biologically active components in *Cordyceps militaris* mushroom mycelia in gestation and lactation diets may affect the performance and health of sows and their piglets. This study, therefore, aimed to evaluate the effects of supplementing *Cordyceps* mushroom mycelia in the diets of pregnant sows on the productivity of the sows and their suckling piglets at the first week of age.

MATERIALS AND METHODS

The experiment was carried out at the Pig's Farmer Farm in Tien Giang province, Viet Nam during March-July 2023.

Ethical regulation

The precent study was approved by the Scientific Comittee of the Faculty of Veterinary Medince & Animal Sciences, HUTECH University; Date: October 25, 2023.

Experimental design

Total pregnant 30 F1 (Landrace × Yorkshire) sows at 2-3 litters were randomly allocated to one of 5 dietary treatments, namely Control, in which the sows were fed the basal diet (Table 1); and T30, T50, T100 and T200, in which, animals were fed a basal diet, and supplemented with 30, 50, 100 and 200 g *Cordyceps militaris* spent mushroom substrates (CMM), respectively. The pregnant sows were individually kept in pens with water and feed supplying systems. They were fed regularly according to the farm procedure.

The cultured *Cordyceps* mushroom mycelia (CMM) used in this study originated from VINABIOMUSH Vietnam Biological Mushroom Limited Company. The CMM comprised the corpus and substrates, which included ingredients such as brown rice, bean sprouts, coconut juice, and silkworm pupae. These ingredients, added to the culture medium, provided nutrients for the *Cordyceps* fungus to grow. After harvesting the fruiting bodies, the CMM was dried and crushed into small particles prior to mixing with feed ingredients. The contents of amino acids, fatty acids, and bioactive compounds in the CMM are presented in Table 2.

Measurements

Performance

In the piglets, total piglet number born, born alive and stunted (less 800 g/pig) piglets; total litter weight at birth and at 7-day old, average daily weight gain of total litter and an individual piglet at the first week of age. In the sows: Daily feed intake during the experiment was recorded. Energy released from milk of the sow was calculated using National Research Council (2012): GE (kcal/kg) = (4.19 x ADG) – (90 x L)

Which, ADG: average daily gain of the litter (g); L: heads of piglets in the litter

Health status

In the piglets, diarrheal rate is total days that piglet got diarrhea incidence divided by total days that piglets raising; Rates of arthritis and painful hoof are proportion between number of piglets got arthritis or painful hoof and total piglet number. In the sows, main health problems such as metritis, missing fetus or remaining placenta, hoof pain, poor milking, mastitis, diarrhea and some disorders without known cause were daily recorded.

Chemical analysis

Samples of *Cordyceps* spent mushroom substrates were collected from VINABIOMUSH, and analyzed at the Quality Assurance & Testing Centre 3 (QUATES 3), District 1, HCMC, Viet Nam. Amino acid composition was analyzed by Performic Acid Oxidation with Acid Hydrolysis–Sodium Metabisulfite Method (AOAC 994.12; 1997). Fatty acid compositions were analyzed by Gas Chromatography of Fatty Acid Methyl Esters Method (ISO 2017). Adenosine and cordycepin concentrations were analyzed by HPLC and detection was performed with a variable-wavelength UV detector at 260 nm.

Statistical analysis

Data were presented in the form of the mean (M). The data were statistically processed by analysis of variance (ANOVA) by General Linear Model in Minitab version 17.2. The difference between the mean values was determined by the Tukey method at a confidence level of 95%. Statistical model: $Y_{ij} = \mu + T_i + e_{ij}$

Where: μ is the average value; T_i is the effect of dietary treatments; e_{ij} is the experimental error.

Table 1 – Ingredients and nutritive values of diets for the sow

Parameters \ Types of animals	Gestation	Lactation
Ingredients (%)		
Maize meal	50.0	46.4
Rice bran	33.4	28.5
Soybean meal	8.0	15.0
Fishmeal	5.0	5.0
Oil	2.0	3.5
Lysine	0.08	0.08
Methionine	0.02	0.02
Premix minerals	0.5	0.5
Premix vitamins	0.5	0.5
Salt	0.5	0.5
Nutritive value (%) *		
Dry matter	89.2	89.4
ME (kcal/kg)	3,040	3,190
Crude protein	14.5	16.5
Crude fibre	10	6
Ca	0.9	1.0
P	0.6	0.8
Lysine	0.8	0.9
Methionine + Cysteine	0.6	0.6

*= Calculated

Table 2 - Amino acid and main fatty acid composition of Cordyceps mushroom mycelia*

Amino acids	mg/g DM	Fatty acids	% as total fatty acids
Histidine	2.15	Palmitic acid (C16:0)	22.4
Arginine	4.47	Oleic acid (C18:3)	37.7
Threonine	3.66	Stearic acid (C18:0)	4.36
Valine	5.32	Linoleic acid (C18:2 n-6)	31.6
Methionine	2.38	Alpha-linoleic acid (ALA, C18:3 n-3).	0.96
Lysine	2.80	Cis-11-Eicosenoic acid (C20:1)	0.39
Isoleucine	6.75	Arachidic acid (C20:0)	0.81
Aspartic acid	8.11	Behenic acid (C22:0)	0.34
Phenylalanine	3.89	Arachidonic acid (C20:4)	0.22
Glutamic acid	13.80		
Alanine	5.09		
Glycine	4.36		
Tyrosine	2.66	Bioactive coumpounds	mg/kg
Proline	4.75	Adenosine	2.60
Serine	3.75	Cordycepin	7.27
Total amino acid	77.70		

*Analysed by QUATEST 3

RESULTS

Performance

Total piglets born and alive were affected by diets (Table 3). The number of piglets born in the Control and T30 was lower than in T50 and T100 ($p<0.05$) but not significant different with T200 ($p>0.05$). The stunted rates were higher in the T100 and T200 than in the Control and T30; however, no stunted piglet born was found in the T50. Number of 7-day old piglets and the survival rate at 7th day old wasn't different among treatments ($p>0.05$).

In Table 4, total litter live weight at birth, at 7th day old and average daily gain (ADG) of the litter were not significantly different between treatments ($p>0.05$). However, the ADG per piglet at the first week of age were higher in T30, T50 and T100 than in T200 ($p<0.05$). In the sows, daily feed intake was significantly lower in T30 than in the other treatments ($p<0.05$). Additionally, the values of GE in milk produced by the sows were significantly higher in Control, T30, T50 and T100 than in T200 ($p<0.05$).

Health status

Piglets

The ratio of suckling piglets got diarrhea was highest in the Control and T30 and lowest in the T100 and T200 ($p<0.05$), and ratio of arthritis was higher in the Control than in T30, T100 and T200 ($p<0.05$).

Lactating sows

In Table 6, the sows got significantly some health problems after farrowing among different treatments. The sows in the Control got more the symptoms of metritis, missed fetus, pain in hoof, matitis, diarhea and fever with un-known cause, meanwhile the sows in the T30, T50, T100 and T200 got less the symptoms than in the Control, excep for painful hoof in T30 and fever and loss of appetite with unknown cause in T30, T50 and T100.

Table 3 - Number of piglets at born and at 7th day of age

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Total piglet born (head)	14.1^{b}	13.6^{b}	15.8^{a}	17.6^{a}	15.2^{ab}	0.049
Piglet born alive (head)	13.1^{ab}	11.9^{b}	14.6^{ab}	15.9^{b}	14.0^{ab}	0.045
Stunted piglets (head)	0.2	0.2	0	0.58	1.0	0,065
Stunted piglet rate (%)	1.12^{b}	1.67^{b}	0	4.93^{a}	3.42^{a}	0.021
Total piglets at 7th day (head)	11.9	10.4	12.5	13.3	12.7	0.059
Survival rate at 7th day (%)	92.47	90.79	87.13	88.53	94.03	0.575

*a,b; Means within a column with different superscripts differ significantly ($P<0.05$)

Table 4 - Total litter weight and average daily gain (ADG), and estimated milk gross energy (GE) produced by the sow at 1st lactation week

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Total litter birth weight (kg)	21.57	17.83	19.51	22.03	20.70	0.318
Total litter weight at 7th day old (kg)	36.42	32.43	34.13	37.41	31.05	0.233
ADG 0-7 (kg/litter)	2.12	2.09	2.09	2.20	1.48	0.742
ADG 0-7 (g/piglet)	180^{ab}	202,9^{a}	200^{a}	195,7^{a}	135,7^{b}	0,002
Daily feed intake (kg/sow)	3.21^{a}	2.27^{b}	3.43^{a}	3.47^{a}	2.71^{ab}	0.046
Milk GE 0-7 (kcal/day)#	7815^{a}	7821^{a}	7631^{a}	8022^{a}	5061^{b}	0.037

*a,b: Means within a column with different superscripts differ significantly ($P<0.05$). Gross Energy: (4.19 x ADG x 1000) – (90 x L), in which L: number of piglets, #: according National Research Council (2012)

Table 5 - Piglets suffering from diarrhea, arthritis and painful hoof (% as total)

Items \ Treatments	Control	T30	T50	T100	T200	p-value
Diarrhea	0.20^{a}	0.20^{a}	0.04^{b}	0.08^{c}	0.09^{c}	0.012
Arthritis	5.05^{a}	2.88^{b}	3.40^{b}	2.15^{b}	2.36^{b}	0.026
Painful hoof	8.43	5.77	6.81	7.53	10.16	0.462

*a,b; Means within a column with different superscripts differ significantly ($P<0.05$)

Table 6 – Sows suffering from diseases after farrowing (% as total)

Items / Treatments	Control	T30	T50	T100	T200	p-value
Metritis	33.4[a]	16.7[b]	0	0	0	Sig
Missed fetus/remaining placenta	16.7	16.7	0	0	0	NS
Painful hoof	33.4	33.4	0	0	0	NS
Poor milk lactating	16.7	0	0	0	0	Sig
Mastitis	16.7	0	0	0	0	Sig
Diarrhea	16.7	0	0	0	0	Sig
Fever, loss of appetite without known cause	50.1[a]	50.1[a]	50.1[a]	50.1[a]	16.7[b]	Sig

*a,b: Means within a column with different superscripts differ significantly (P<0.05).

DISCUSSION

Chemical composition and bioactive compounds

In recent years, knowledge of the full chemical composition, bioactive compounds and nutritive values of cultured *C. militaris* has been studied (Ji et al., 2020; Sharma et al., 2024; Trung et al., 2024). In the framework of research of Hur (2008) and Chan et al. (2015), the proximate composition and content of amino acids, fatty acids, elements, vitamins, and bioactive compounds of the fruiting body (FB) and mycelial biomass or corpus (MB) of *C. militaris* were studied. The authors found significant differences in some important parameters of the chemical composition between FB and MB. In general, the MB contained valuable nutrients 15-20% compared to the FB such as the contents of free amino acids, some fatty acids and bioactive compounds of the FB were much higher than in MB. In addition, the MB were much cheaper than the FB.

In this study, the content of total free amino acids in the CMM is 77.7 mg/g DM (Table 2), and the most abundant amino acids are glutamic acid, aspartic acid and isoleucine. Meanwhile, the contents of lysine and methionine are low as compared with other amino acids. The content of total free amino acids in the study is ranged between the previous once. In the previous reports, the content of free amino acids in the corpus was 14.03 mg/g (Hur, 2008) and in the mycelial biomass was 24.98% as DM or 249.8 mg/g DM (Chan et al., 2015). Furthermore, Hur (2008) reported also that the most abandon amino acids in the corpus were proline (2.99 mg/g DM), lysine (2.2 mg/g DM) and glutamic acid (1.4 mg/g DM). It is not in case of Chan et al. (2015), who has found the most abandon amino acids in the mycelial biomass were alanine (3.61%), glycine (3.12%) and arginine (2.76%), and low contents of glutamic acid (1.82%) and aspartic acid (1.92% as DM).

Regarding to the content of fatty acids, the most abandon fatty acids in the CMM in this study are palmitic acid (22.4%), oleic acid (37.7%) and linoleic acid (31.6%) and low alpha-linolenic acid (0.96%). In our study, the content of linoleic acid (LA) is higher than alpha-linolenic acid (ALA). This finding is agreement with the study of Hur (2008) and Chan et al. (2015). The contents of LA and ALA in the corpus were 33% and 20.6% as total fatty acid, respectively (Hur, 2008) and LA of 40.7% and ALA 0.9% as total fatty acid in the mycelium (Chan et al., 2015).

The contents of bioactive compounds as adenosine and cordycepin in this study were 2.60 mg/g DM and 7.27 mg/g DM, respectively (Table 2). Cronstein et al. (1994) indicated that adenosine inhibits some functions of neutrophils, which are a type of inflammatory cell in human being. Cordycepin is an adenosine derivative, and was shown to possess diverse pharmacological properties, in addition to its well-known antioxidant effects (He et al., 2013; Olatunji et al., 2016), including antiviral activity (Ryu et al., 2014), inhibition of lipopolysaccharide (LPS)-induced inflammation (Kim et al 2006), reduction of blood lipids (Guo et al 2010), inhibition of platelet aggregation (Cho et al., 2007), and induction of apoptosis in neuroblastoma and melanoma cells (Baik et al., 2007) in human being. In the previous study, Hur (2008) shown the contents of adenosine and cordycepin in the corpus were 0.06% (0.6 mg/g) and 0.36% (3.6 mg/g), respectively. In the mycelial biomass, the content of cordycepin was 0.182% or 1.82 mg/g (Chan et al., 2015) and 1.74 mg/g (Cohen et al., 2014). Therefore, the content of cordycepin in our recent study was higher than those mentioned above. Some studies reported that the concentration and distribution of bioactive compounds is not uniform event in the fruiting bodies. Additionally, the optimal drying temperature for *C. militaris* is 60°C and higher temperature causes a loss of the content of cordycepin and phenolic compounds (Wu et al., 2019).

Performance and health status

In this study, the supplementation of the CMM in diets for pregnant and lactating sows improved the litter size, the health of suckling piglets and the sows, and didn't affect growth rate of piglets and milk energy released from the sows except when the sow got 200 g CMM/day. As calculation, the sows in T30, T50, T100 and T200 got 2.18; 3.64; 7.27 and 14.54 mg cordycepin per day, respectively. Ahtwichai et al. (2019) reported that supplementing 2.45 mg and 4.9 mg of cordycepin per day in the diets of gestation and lactating sows didn't affect the sow's productivity and piglet's production parameters but affected litter fecal score and wean to estrus interval.

In human being, the inclusion of *C. militaris* extract in the diet can have several health benefits. However, a single substance may have a less therapeutic effect and may not show synergistic effects with other compounds present in mushroom. Among all bioactive substances mentioned in the studies, the greatest therapeutic potential is associated with cordycepin (Jedrejko et al., 2021). Additionally, understanding the production modes and metabolite yields of *C. militaris* is crucial for realizing its full potential in medicinal and industrial applications (Chou et al., 2024).

According to Boontiam et al. (2020b), suckling piglets received a diet supplemented with cordyceps, had secrete more IgA and IgG. In addition, piglets received IgG through colostrum periodically through an intestinal transport mechanism that operated specifically during the first 24 hours after birth allowing the delivery of maternal IgG into the intestine via the circulatory system. In addition, this stimulation may be that cordyceps, originating from the Thai rice medium, contained large amounts of γ-oryzanol, which has been shown to have a potential effect on immunity, by way to activate IgA production (Yang et al., 2014; Henderson et al., 2012).

Cordyceps militaris is effective in reducing the amount of pathogenic *E. coli* negative gram bacteria and increasing the amount of probiotic *Lactobacillus* spp. may be due to the abundance of polysaccharides (Yu et al., 2004). Galactomanna is the main polysaccharide antigen (mannooligosaccharides: MOS) in *C. militaris* and is useful for prebiotic production. A study by Kudoh et al. (1999) on mice, IgA levels increased in the feces of mice supplemented with MOS, IgA secretion can be regulated by the presence of bacterial antigens in the intestine and IgA plays a role in important for mucosal immunity because it inhibits bacterial adhesion and invasion, which further reduces bacterial colonization. In addition, according to Farmer and Quesnel (2009), IgA titers increased in sow milk. Therefore, the difference in the rate of piglet diarrhea days can be explained that adding *Cordyceps* to the diet helps reduce the rate of piglet diarrhea, which is in agreement with the study of Omthonglang et al. (2021). In addition, the results reported by Boontiam et al. (2020b), *C. militaris* can be used to control diarrhea because it has the ability to reduce the amount of *E. coli* in feces in weaned pigs. Additionally, serum immunoglobulin is an important index to determine humoral immunity in pigs. IgA and IgG play an important role in protecting against invading pathogens, so changes in the levels of these proteins can affect the growth performance and immunity of pigs (Hedegaard et al., 2016). Cordycepin and polysaccharides in fermented *Cordyceps* corpus are considered prebiotics in animal nutrition (Chuang et al., 2020; Sun et al., 2021). Prebiotics promote the growth of beneficial bacteria and enhance the absorption of nutrients, helping to reduce the amount of harmful metabolic products in the intestines and stimulating the growth of useful bacteria.

CONCLUSION

Supplementation of *Cordyceps* mushroom mycelia in the diets for the pregnant and lactating sows affected:

- Total piglets born and alive, average daily gains per piglet at the first week of age but not the survival rate of 7-day old piglets;
- Daily feed intake and the values of gross energy in milk produced by the sows;
- The health of both piglets and their mothers.

In conclusion, the supplementation of 50-100 g dried *Cordyceps* mushroom mycelia in the pregnant and lactating sows' diets improved performance and health status of both the mothers and their piglets.

DECLARATIONS

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Author contributions

Nguyen Vu Thuy Hong LOAN and Do Ngoc Yen PHUONG conceived, designed the experiments; Do Ngoc Yen PHUONG performed the experiments; Nguyen Vu Thuy Hong LOAN analysed the data; Nguyen Vu Thuy Hong LOAN and wrote the paper; all authors reviewed and approved the final manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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