



# Effects of seeding dates on dry matter yield and feed values of whole-crop barley cultivated in a paddy field

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## Abstract

This study was carried out to investigate the effects of seeding dates on the yield and feed value of whole-crop barley cultivated in paddy soil. The field experiment was conceived as a randomized block design performed in triplicate with seeding dates of Oct. 19 (T1 as treatment 1), Oct. 26 (T2 as treatment 2), Nov. 2 (T3 as treatment 3), Nov. 9 (T4 as treatment 4), and Nov. 16 (T5 as treatment 5) as treatments. The barley grown in all treatments was harvested on May 21 of the following year. Plant length, fresh yield, dry matter yield, and total digestible nutrient (TDN) yield were higher ( $p < 0.05$ ) in barley with early seeding dates, whereas crude protein and ether extract were higher ( $p < 0.05$ ) with late seeding dates. Crude fiber and neutral detergent fiber were highest in the T3 treatment and lowest in the T1 treatment. There was no significant difference between acid detergent fiber and TDN among the different seeding dates. Total mineral contents were higher in the order T1 > T2 > T3 > T4 > T5 ( $p < 0.05$ ), whereas the total amino acid content increased significantly ( $p < 0.05$ ) with a delay in seeding date. Free sugar contents (fructose, glucose, and sucrose) were higher with early seeding dates ( $p < 0.05$ ). Collectively, the results obtained in this study indicate that it is favorable to seed soon after harvesting rice to increase dry matter and TDN yields and mineral and free sugar contents of whole-crop barley in the midlands of Korea.

**Keywords:** Whole-crop barley, Seeding dates, Dry matter yield, Total digestible nutrients

## Background

With the continuing steep increase in the number of ruminant animals, mainly beef cattle, reared on farms, there has been a concomitant increase in the demand for forage and concentrate. However, in Korea, because there is a limited production capacity for forage and grains, dependency on foreign imports has been rising. The price of imported hay and grain can fluctuate markedly, depending on international supply capacity, and under such circumstances it is difficult for farmers to continue rearing livestock [1]. Therefore, to supply home-produced meat to customers in Korea, it is imperative to expand forage production capability to meet the demand for

ruminant production. In a country like Korea with limited agricultural land area, using agricultural land that remains idle after rice crop cultivation accordingly represents a very important approach for increasing forage production [2].

A forage crop suitable for this purpose is whole-crop barley, which has strong moisture tolerance when grown in paddy fields [3], a high feed value [4], and high quality silage, which makes it suitable as a winter crop [5]. In addition, whole-crop barley can improve feed efficiency and meat quality, and reduce feed costs compared with rice straw [6,7]. There are several reports available in the literature describing new varieties of whole-crop barley that are adaptable to Korean agricultural land [8–10], and good in

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terms of dry matter yield and nutritive value [4,7,11], and seeding method and rate [12]. However, to date, there have been no reported studies on the mineral contents, amino acid compositions, and free sugar contents of these varieties. Therefore, this study was conducted to investigate the effects of different seeding dates on the dry matter yield, total digestible nutrients (TDN), and compositions of minerals, amino acids, and free sugars when forage barley was seeded in a paddy field after harvesting a rice crop. The results of this study will provide livestock producers and/or forage makers with fundamental information on appropriate seeding dates for cultivation of winter barley in paddy fields.

## Materials and Methods

### Experimental design, seeding, and forage yield

This field experiment was performed from October 19, 2009 to May 21, 2010 in a paddy field in the northwestern region of Gyeongbuk Province (latitude 36.5592, longitude 128.1885), Korea. The field experiment employed a randomized complete block design with five different seeding dates as experimental treatments: Oct. 19 (T1 as treatment 1), Oct. 26 (T2 as treatment 2), Nov. 2 (T3 as treatment 3), Nov. 9 (T4 as treatment 4), and Nov. 16 (T5 as treatment 5). The barley grown in all treatments was harvested on May 21 of the following year. The whole-crop barley cultivar used for the experiment was “Yuyeon,” which was developed in Korea [13]. The paddy field used for the field experiment had higher organic matter and nitrogen content and lower phosphate content than a general upland soil, as previously reported by Lee [14]. The application rates of chemical fertilizer were as follows: total nitrogen (200 kg/ha), phosphorus (150 kg/ha), and potassium (150 kg/ha). The chemical fertilizer was applied with 40% nitrogen and potassium used as a basal fertilizer and 60% as added fertilizer, whereas total phosphorus was applied as a basal fertilizer. As a seeding method, the whole-crop barley was broadcasted on the soil at a rate of 180 kg/ha. The experimental plot area was 3 m × 5 m = 15 m<sup>2</sup>. Fresh forage yield was estimated after cutting 5 m<sup>2</sup> in each replicate.

### Analysis of chemical composition

The samples were dried for 3 days at 55 °C and used for analysis. The chemical composition of the samples was determined by using the method specified by the Association of Analytical Chemists (AOAC) [15]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed using the methods of AOAC [16] and the Goering and Van Soest method [17], respectively. The TDN was estimated using the equation of Moore and Undersander [18]:  $TDN = 88.9 - (0.79 \times ADF)$ . The mineral composition was analyzed from pre-treated samples using inductively coupled plasma mass spectrometry (Iris Intrepid, Thermo Elemental Co., UK).

Analysis of the amino acid profile was performed in the following sequence. To 1 g of accurately weighed pulverized sample in a test tube, 10 mL of 6 N HCl was added. Following a reduction in pressure, the tube was sealed and the sample was then subjected to hydrolysis at 110 °C in a drying oven for 24 h. The pre-treated sample was then filtered through a 0.45-µm membrane filter, and the filtered sample was analyzed using an automatic amino acid analyzer (Biochrom 30, Biochrom Ltd, Cambridge, England). Free sugar was analyzed using the method of Wilson et al. [19]. Briefly, 100 mL of 80% ethanol solution was added to 5 g of sample. The sugar composition was then extracted repetitively for 2 h at 80 °C using the heating mantle in a reflux cooling extraction unit followed by filtering through Whatman No. 5 filter paper. The resulting solution was then analyzed using high-performance liquid chromatography (Waters 2414, Waters Co., USA).

### Statistical analysis

The results were subjected to one-way analysis of variance with seeding date as a main effect. Mean values and standard deviations of the experimental results were obtained using SAS [20]. Duncan's multiple range test was employed to identify differences among the treatments, which were considered significant when  $p < 0.05$ .

## Results and Discussion

### Growth characteristics and dry matter yield

The growth characteristics according to seeding date are shown in Table 1. Plant length varied in the following order T1 > T2 > T3 > T4 > T5 ( $p < 0.05$ ). Consistent with the findings of Kim et al. [12], it was shown that the plant length of whole-crop barley became shorter with an increasing delay in seeding date. Stem diameter increased significantly until the grain was formed (i.e., T5 < T4 < T3); however, it decreased with maturation of the grain (T3 > T2 > T1). Fresh and dry matter yields were higher when the seeding date was earlier ( $p < 0.05$ ), and there was a notable trend of rapidly decreasing yield with the progression of November seeding dates (T3, T4, and T5). The results of this study are in line with those reported by Kim et al. [2] and Suh [21], who demonstrated that when cultivating winter crops after rice cultivation, there was a difference in yield according to the seeding date. Kim et al. [2] reported that when seeding date is delayed in autumn, the average temperature drops and average winter survival rates rapidly decrease, resulting in a reduced dry matter yield. In the present study, it was shown that TDN yield was significantly higher ( $p < 0.05$ ) in T1 than in the other treatments, particularly T3, T4, and T5, with late seeding dates being associated with a very low yield ( $p < 0.05$ ). These results are consistent with those reported by Kim et al. [2],

**Table 1.** Effect of seeding dates on the growth characteristics and yield of whole-crop barley cultivated in a paddy field

Items	Treatments <sup>1)</sup>				
	T1	T2	T3	T4	T5
Plant length (cm)	101.4 ± 2.5 <sup>a</sup>	96.2 ± 3.1 <sup>b</sup>	94.6 ± 2.1 <sup>b</sup>	87.8 ± 3.1 <sup>c</sup>	80.6 ± 1.7 <sup>d</sup>
Stem diameter (mm)	5.5 ± 0.2 <sup>bc</sup>	5.9 ± 0.1 <sup>a</sup>	6.0 ± 0.2 <sup>a</sup>	5.7 ± 0.3 <sup>ab</sup>	5.2 ± 0.2 <sup>c</sup>
Fresh yield (kg/ha)	50,514 ± 1,112 <sup>a</sup>	32,700 ± 2,321 <sup>b</sup>	25,426 ± 2,807 <sup>c</sup>	20,792 ± 1,446 <sup>d</sup>	8,729 ± 1,558 <sup>e</sup>
Dry matter yield (kg/ha)	9,952 ± 218 <sup>a</sup>	7,390 ± 524 <sup>b</sup>	5,594 ± 459 <sup>c</sup>	4,636 ± 322 <sup>d</sup>	1,947 ± 347 <sup>e</sup>
TDN yield(kg/ha)	5,987 ± 130 <sup>a</sup>	4,399 ± 312 <sup>b</sup>	3,328 ± 273 <sup>c</sup>	2,754 ± 192 <sup>d</sup>	1,148 ± 205 <sup>e</sup>

Data are expressed as means ± SD.

Means in a row with different superscripts are significantly different (*p* < 0.05).

<sup>1)</sup>T1, T2, T3, T4, and T5 represent the different seeding dates: October 19, October 26, November 2, November 9, and November 16, respectively.

TDN, total digestible nutrients, yield = [88.9 – (0.79 × acid detergent fiber (%))] × dry matter yield/ha.

who showed that TDN and dry matter yield increased with earlier seeding dates in winter crops. Therefore, to increase the yield of whole-crop barley in the Korean midlands after rice cultivation, it is favorable to seed soon after the rice has been harvested.

**Chemical composition**

The chemical composition of whole-crop barley according to seeding date is presented in Table 2. The crude protein and ether extract contents of whole-crop barley decreased significantly with an earlier seeding date (*p* < 0.05), whereas crude ash content increased with an earlier seeding date (*p* < 0.05). NDF increased until the time of grain formation time; however, it decreased during the grain maturity stage (*p* < 0.05). This is presumably because as the cultivation period increases, maturity stages develop, the leaf ratio of the plant decreases, and stems harden [2,22]. There was no significant difference between the ADF and TDN content in relation to seeding date. The chemical compositions of forage crops differ significantly, depending on cutting time [23], maturity stage [24], variety [2], seeding date [2], and manure conditions [25]. In

the present study, feed value appeared to be high in the late seeding groups (T4 and T5); however, as shown in Table 4, when the seeding date is late, there is a significant decrease in yield. Therefore, seeding dates should be determined by taking into consideration the requisite dry matter yield and nutritional value.

**Mineral contents**

The mineral contents of whole-crop barley according to seeding date is presented in Table 3. Irrespective of seeding date, the mineral contents of whole-crop barley were higher in the order K > Ca > Na > Mg. A similar trend has been reported by Lee [25] and Lee [14]. The Ca, K, and Na contents were highest in T1 (*p* < 0.05), whereas Fe content was highest in T2 and P content was highest in T4 (*p* < 0.05). Total mineral content was highest for seeding date T1 (*p* < 0.05) at 31,308 mg/kg and lowest for T5 (*p* < 0.05) at 15,978 mg/kg. These values are lower than those reported by Kim and Lee [1], who determined mineral contents of between 39,027 and 51,914 mg/kg for whole-crop barley varieties. Generally, it is known that mineral content is affected by a range of conditions,

**Table 2.** Effects of seeding dates on the chemical composition of whole-crop barley cultivated in a paddy field (% of dry matter basis unless otherwise stated)

Items	Treatments <sup>1)</sup>				
	T1	T2	T3	T4	T5
Crude protein	12.2 ± 0.2 <sup>c</sup>	12.7 ± 0.1 <sup>c</sup>	13.5 ± 0.1 <sup>b</sup>	13.8 ± 0.2 <sup>b</sup>	15.4 ± 0.6 <sup>a</sup>
Ether extract	1.8 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>b</sup>	1.9 ± 0.0 <sup>b</sup>	2.1 ± 0.0 <sup>a</sup>	2.1 ± 0.1 <sup>a</sup>
Crude ash	9.7 ± 0.1 <sup>a</sup>	9.1 ± 0.1 <sup>b</sup>	8.9 ± 0.1 <sup>bc</sup>	9.0 ± 0.1 <sup>bc</sup>	8.8 ± 0.1 <sup>c</sup>
Crude fiber	28.5 ± 0.4 <sup>b</sup>	30.9 ± 0.2 <sup>a</sup>	31.1 ± 0.7 <sup>a</sup>	30.5 ± 0.3 <sup>a</sup>	30.9 ± 1.3 <sup>a</sup>
NDF	58.6 ± 0.3 <sup>b</sup>	59.9 ± 0.2 <sup>a</sup>	61.8 ± 0.5 <sup>a</sup>	60.5 ± 0.7 <sup>a</sup>	60.2 ± 0.7 <sup>a</sup>
ADF	36.9 ± 1.2 <sup>ns</sup>	37.5 ± 0.8	37.2 ± 2.6	37.3 ± 1.5	37.7 ± 0.9
TDN	60.0 ± 1.0 <sup>ns</sup>	59.5 ± 0.3	59.3 ± 2.1	59.4 ± 1.2	59.0 ± 0.7

Data are expressed as means ± SD.

Means in a row with different superscripts are significantly different (*p* < 0.05).

<sup>1)</sup>T1, T2, T3, T4, and T5 represent the different seeding dates: October 19, October 26, November 2, November 9, and November 16, respectively.

NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients [88.9 – (0.79 × acid detergent fiber (%))]; ns, not significant.

**Table 3.** Effects of seeding dates on the mineral contents of whole-crop barley cultivated in a paddy field (mg/kg dry matter basis unless otherwise stated)

Items	Treatments <sup>1)</sup>				
	T1	T2	T3	T4	T5
Ca	10,102 ± 242 <sup>a</sup>	10,722 ± 360 <sup>a</sup>	9,260 ± 203 <sup>b</sup>	5,626 ± 63 <sup>c</sup>	4,992 ± 180 <sup>d</sup>
Cu	4 ± 0 <sup>a</sup>	4 ± 0 <sup>a</sup>	4 ± 0 <sup>a</sup>	3 ± 0 <sup>b</sup>	3 ± 0 <sup>b</sup>
Fe	348 ± 7 <sup>b</sup>	427 ± 25 <sup>a</sup>	352 ± 13 <sup>b</sup>	198 ± 15 <sup>c</sup>	182 ± 14 <sup>c</sup>
K	18,568 ± 361 <sup>a</sup>	15,015 ± 946 <sup>b</sup>	9,461 ± 1,177 <sup>c</sup>	9,434 ± 542 <sup>c</sup>	9,561 ± 621 <sup>c</sup>
Mg	479 ± 23 <sup>b</sup>	679 ± 9 <sup>a</sup>	754 ± 44 <sup>a</sup>	526 ± 32 <sup>b</sup>	374 ± 71 <sup>c</sup>
Mn	26 ± 1 <sup>b</sup>	29 ± 2 <sup>a</sup>	25 ± 2 <sup>b</sup>	19 ± 2 <sup>c</sup>	21 ± 2 <sup>c</sup>
Na	1,370 ± 103 <sup>a</sup>	822 ± 21 <sup>b</sup>	747 ± 21 <sup>b</sup>	459 ± 7 <sup>c</sup>	256 ± 13 <sup>d</sup>
Zn	18 ± 3 <sup>c</sup>	23 ± 2 <sup>a</sup>	23 ± 1 <sup>ab</sup>	20 ± 2 <sup>bc</sup>	18 ± 1 <sup>c</sup>
P	392 ± 38 <sup>c</sup>	371 ± 15 <sup>c</sup>	408 ± 30 <sup>c</sup>	648 ± 21 <sup>a</sup>	571 ± 37 <sup>b</sup>
Total	31,308 ± 501 <sup>a</sup>	28,093 ± 1,206 <sup>b</sup>	21,033 ± 1,028 <sup>c</sup>	16,934 ± 501 <sup>d</sup>	15,978 ± 582 <sup>d</sup>

Data are expressed as means ± SD.

Means in a row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1)</sup>T1, T2, T3, T4, and T5 represent the different seeding dates: October 19, October 26, November 2, November 9 and November 16, respectively.

including those of weather, soil, and fertilization [26–28].

Interestingly, information on the mineral contents of whole-crop barley cultivars and other domestically produced forages (i.e., fresh or conserved Italian ryegrass) that have been developed in Korea is extremely limited in the literature, and consequently it is difficult to compare our results with published data. There may be several reasons for the lack of information regarding mineral contents. Firstly, this is presumably attributable to the difficulty in performing the analytical procedures necessary to assess individual mineral contents, as they are expensive and laborious compared with those of the simple proximate analyses commonly performed in feed analytical laboratories [29]. Secondly, the quantity of domestically produced fresh or conserved forages as a forage source to supply nutrients to ruminants has traditionally been limited, and therefore the mineral contents of these forages have seldom been considered as a significant ingredient when formulating diets for ruminants. However, this latter possibility may no longer pertain because forage production within Korea has been encouraged by the central government, and production has been steeply increasing over the last few years [2]. Therefore, for farmers and feed formulators, as well as for consultants, information such as the mineral contents of domestically produced feed resources will become immensely important.

### Amino acids profile

The amino acid compositions of whole-crop barley according to seeding date are presented in Table 4. In terms of essential amino acids, there was no difference among treatments. The total essential amino acid content showed a trend of T4 > T3 > T5 > T2 > T1, although no significant difference was noted. For non-essential amino acids, all treatment groups had high glutamic acid, proline, and

aspartic acid contents. The non-essential amino acid content trend was T5 > T4 > T3 > T2 > T1 ( $p < 0.05$ ). The range of total amino acids by seeding date was from 7,066 to 7,747 mg/100 g. These values are higher than those (5,971–6,499 mg/100 g) reported by Kim and Lee [1]. The total amino acid content was highest in T5 and lowest in T1. Such differences in the levels of total amino acids are considered to be attributable to differences in crude protein content, as shown in Table 2. Similar to mineral contents, information on total and individual amino acids, including essential amino acids, is rare for home-grown forages in Korea. This is of particular concern in the dairy sector, in which the concept of rumen degradable and rumen undegradable protein is adopted to formulate diets for high-producing dairy cows in Korea. Thus, considerable information on amino acid profiles is required for domestically produced forages. In this regard, our laboratory has previously reported on the amino acid profiles of corn silage [30] and sorghum × sudangrass hybrids [31].

### Free sugar contents

The free sugar contents in whole-crop barley were in the order sucrose > glucose > fructose (Table 5). In terms of individual sugar contents, T1 had the highest content and T5 had the lowest when expressed per 100 g dry matter basis ( $p < 0.05$ ). Although the free sugar content is high at the early stage of grain maturation, it decreases as the grain matures [32]. The highest free sugar content of T5 appeared to be related to the stage of maturity (harvest of the milk stage). Generally, the free sugar content of forage crops is a very important ingredient because it affects silage fermentation [25,28]. In particular, a higher free sugar content is associated with a decreased rate of butyric acid and ammonia-N production and increased lactic acid yield, through enhancement of the rate of mi-

**Table 4.** Effects of seeding dates on the amino acid profile of whole-crop barley cultivated in a paddy field (mg/100 g dry matter basis unless otherwise stated)

Items	Treatments <sup>1)</sup>				
	T1	T2	T3	T4	T5
Threonine	268 ± 14 <sup>ns</sup>	260 ± 10	269 ± 6	272 ± 2	262 ± 8
Valine	460 ± 26 <sup>ns</sup>	458 ± 12	482 ± 4	504 ± 43	518 ± 15
Methionine	34 ± 1 <sup>ns</sup>	33 ± 1	35 ± 3	33 ± 3	39 ± 3
Isoleucine	298 ± 15 <sup>ns</sup>	291 ± 9	312 ± 5	310 ± 8	304 ± 11
Leucine	504 ± 25 <sup>ns</sup>	490 ± 10	511 ± 3	508 ± 3	482 ± 4
Phenylalanine	450 ± 6 <sup>ns</sup>	478 ± 12	490 ± 7	489 ± 14	476 ± 30
Histidine	218 ± 2 <sup>ns</sup>	226 ± 6	238 ± 2	248 ± 3	254 ± 30
Lysine	406 ± 26 <sup>ns</sup>	410 ± 11	428 ± 7	428 ± 8	397 ± 12
Arginine	305 ± 11 <sup>ns</sup>	307 ± 5	332 ± 4	326 ± 11	331 ± 20
Sum of EAA	2,943 ± 127 <sup>ns</sup>	2,953 ± 31	3,097 ± 23	3,118 ± 84	3,063 ± 39
Serine	270 ± 9 <sup>ns</sup>	278 ± 7	292 ± 4	296 ± 24	285 ± 26
Glutamic acid	1,086 ± 27 <sup>ns</sup>	1,055 ± 53	1,065 ± 55	1,117 ± 34	1,076 ± 21
Proline	1,035 ± 4 <sup>ns</sup>	924 ± 98	1,138 ± 29	1,148 ± 7	1,140 ± 41
Glycine	299 ± 10 <sup>ns</sup>	293 ± 8	306 ± 6	304 ± 4	289 ± 11
Alanine	339 ± 31 <sup>ns</sup>	406 ± 16	425 ± 6	423 ± 6	408 ± 5
Tyrosine	97 ± 7 <sup>ns</sup>	94 ± 2	102 ± 1	101 ± 1	107 ± 5
Aspartic acid	997 ± 34 <sup>ns</sup>	1,115 ± 48	1,177 ± 6	1,171 ± 61	1,381 ± 24
Sum of NEAA	4,123 ± 101 <sup>c</sup>	4,315 ± 41 <sup>b</sup>	4,505 ± 13 <sup>a</sup>	4,560 ± 52 <sup>a</sup>	4,686 ± 96 <sup>a</sup>
Total amino acids	7,066 ± 26 <sup>b</sup>	7,268 ± 11 <sup>b</sup>	7,602 ± 27 <sup>a</sup>	7,678 ± 136 <sup>a</sup>	7,747 ± 135 <sup>a</sup>

Data are expressed as means ± SD.

Means in a row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1)</sup>T1, T2, T3, T4, and T5 represent the different seeding dates: October 19, October 26, November 2, November 9, and November 16, respectively.

ns, not significant; EAA, essential amino acids; NEAA, non-essential amino acids.

**Table 5.** Effects of seeding dates on the free sugar contents of whole-crop barley cultivated in a paddy field (mg/100 g dry matter unless otherwise stated)

Items	Treatments <sup>1)</sup>				
	T1	T2	T3	T4	T5
Fructose	913 ± 77 <sup>a</sup>	907 ± 64 <sup>a</sup>	572 ± 25 <sup>b</sup>	284 ± 11 <sup>c</sup>	209 ± 15 <sup>c</sup>
Glucose	1,498 ± 62 <sup>a</sup>	1,488 ± 72 <sup>a</sup>	1,371 ± 19 <sup>b</sup>	1,208 ± 24 <sup>c</sup>	1,262 ± 45 <sup>c</sup>
Sucrose	1,878 ± 66 <sup>a</sup>	1,713 ± 44 <sup>b</sup>	1,685 ± 59 <sup>b</sup>	1,508 ± 67 <sup>c</sup>	1,304 ± 21 <sup>d</sup>
Total	4,289 ± 202 <sup>a</sup>	4,108 ± 179 <sup>a</sup>	3,628 ± 59 <sup>b</sup>	3,000 ± 53 <sup>c</sup>	2,775 ± 42 <sup>c</sup>

Data are expressed as means ± SD.

Means in a row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1)</sup>T1, T2, T3, T4, and T5 represent the different seeding dates: October 19, October 26, November 2, November 9, and November 16, respectively.

crobial fermentation. It has also reported that a high sugar content maintains pH at a low level, thereby promoting the rapid stabilization of silage [33].

### Competing interests

No potential conflict of interest relevant to this article was reported.

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### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

### Authors' contributions

Conceptualization: Lee SM.



Data curation: Lee SM.

Formal analysis: Lee SM.

Methodology: Lee SM.

Investigation: Lee SM, Kim EJ.

Writing - original draft: Lee SM, Kim EJ.

Writing - review & editing: Lee SM, Kim EJ.

### Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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