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Fatty Acid Composition of Endangered Land Snail *Koreanohadra koreana* (Mollusca: Gastropoda) of South Korea by Season, Region, and Microhabitat

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ABSTRACT

참달팽이(*Koreanohadra koreana*)는 환경부에서 지정한 법정보호종(멸종위기 야생생물 Ⅱ급)이며, 전라남도 신안군 홍도와 하태도에서만 서식하는 한국고유종이다. 반면, 그 동안 참달팽이 지방산에 대한 연구는 진행된 바 없다. 홍도와 하태도를 대상으로 계절, 미소서식처, 지역 차이에 따른 참달팽이의 지방산 구성 변화를 비교하기 위해 분석에 사용한 표본의 개수는 총 99개였다. 실험결과 총 16가지의 지방산이 검출되었으며, 주로 식물질 먹이에서 유래되는 다중불포화지방산(PUFAs)이 53.6%로 가장 높았다. 반면, 동물성 또는 미생물에서 유래하는 단일불포화지방산(MUFAs)은 31.4%, 포화지방산(SFAs)은 15.0%로 상대적으로 적게 검출되었다. 참달팽이의 총 지방산량 변화는 PUFAs의 변화와 가장 강한 상관관계(R^2=0.88, p < 0.0001)를 보였다. 계절별로 PUFAs는 봄 (13.20 mg/g)과 여름 (13.08 mg/g)이 가을 (12.06 mg/g)과 겨울 (12.40 mg/g)에 비해 많은 양이 검출되었다. 미소서식처별 PUFAs는 초지대(12.80 mg/g)에서 석회질 지대(12.48 mg/g) 보다 다소 높게 나타났다. 반면, 지역별로 홍도(12.65 mg/g)와 하태도(12.64 mg/g)는 차이가 거의 없었다. PUFAs는 참달팽이의 먹이원을 기반으로 한 서식지의 질을 추적할 수 있는 하나의 지표로서 활용가치가 높을 것으로 기대된다.

Key Words: 참달팽이, 먹이원, 지방산, 멸종위기 야생생물, 서식지 질

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I. Introduction

Koreanohadra koreana Pfeiffer (1850) was found only near Hongdo Island, Sinan-gun, Jeollanam-do, South Korea, and is protected by the Ministry of Environment as Class II endangered species. Hongdo Island is a natural monument 170 (April 7, 1965), Dadohae Sea National Park No. 478 (December 23, 1981) and UNESCO Biosphere Reserve (2009.5.26), which has been protected from human activities such as development and illegal poaching etc. Nevertheless, *K. koreana* habitat is exposed to various threat such as climatic ecological factors (temperature, humidity etc.). In particular, changes in vegetation due to climate change can pose greatest threat to survival of *K. koreana* that primarily feed on plant leaves. In addition, Hongdo Island is geographically isolated from inland environment, limiting movement and spread of *K. koreana*. To track long-term changes in habitat quality of *K. koreana*, research on characteristics of their food source should be prioritized.

Determination of habitat of living organisms is very closely related to source of food (Kim et al., 2022). However, it is very difficult to evaluate how each food source affects growth and secondary reproduction of organisms because it is difficult to directly observe feeding process within habitat for a long time (Dame, 1996). Indirect method such as gut content analyses (Kamermans, 1994) also have limitations because they provide snapshot information about ingested foods and not what is assimilated for a long time (Sauriau and Kang, 2000). However, since fatty acid composition of organism reflects fatty acid of ingested food source, it is possible to estimate food source from fatty acid information of organism (Shin and Kim, 2010). In particular, polyunsaturated fatty acids (PUFAs) are closely related to prostaglandin formation, which is directly involved in regulation of reproduction, renal function, and ion regulation, as is known in mollusk species (Stanley-Samuelson, 1987). In addition, ingested bacteria can be used as tracers (Gayoso et al., 1997) because they change little even if fed by consumers (Gayoso et al., 1997). Fatty acids in commercially important marine and freshwater mollusks have been reported and reviewed in various and detailed ways (Ackman, 2000; Karakoltsidis et al., 1995). However, lipid data correlating with nutritional, physiological, structural and environmental factors of land snails are particularly limited in literature and relatively little is known about fatty acid composition of land snails. Therefore, purpose of this study was to secure basic data for habitat conservation by examining fatty acid composition of *K. koreana* and characterizing its food sources.

II. Material and Method

1. Collecting Samples

K. koreana is designated as class II endangered species by the Ministry of Environment. For conservation of *K. koreana*, capture was carried out with permission of the Yeongsangang River Basin Environmental Office (Permission No. 2019-8, 2019-55, and 2020-7). In order to analyze food source of *K. koreana* using fatty acids, a total of 99 samples were collected from June 2019 to September 2020 using visual survey methods in calcareous and grassy habitats with high population density in

Table 1. Number of *K. koreana* samples collected by season, region, and microhabitat

region	microhabitat	season				total
		spring	summer	autumn	winter	
Hongdo	calcareous	-	17	17	5	39
	grassy	-	16	13	1	30
Hataedo	calcareous	6	5	3	10	24
	grassy	1	5	-	-	6
total		7	43	33	16	99

Table 2. 36 types of fatty acids analysis targets

1.	Butyric acid (butanoic acid)	13.	Caproic acid (hexanoic acid)	25.	Caprylic acid (octanoic acid)
2.	Capric acid (decanoic acid)	14.	Undecylic acid (undecanoic acid)	26.	Lauric acid (dodecanoic acid)
3.	Tridecylic acid (tridecanoic acid)	15.	Myristic acid (tetradecanoic acid)	27.	Myristoleic acid
4.	cis-10-Pentadecenoic acid	16.	Palmitic acid (hexadecanoic acid)	28.	Palmitoleic acid
5.	Margaric acid (heptadecanoic acid)	17.	cis-10-Heptadecenoic Acid	29.	Stearic acid (octadecanoic acid)
6.	Elaidic acid	18.	Oleic acid	30.	Linolelaidic acid
7.	Linoleic acid	19.	Gamma-linolenic acid (GLA)	31.	Alpha-linolenic acid (ALA)
8.	Arachidic acid (icosanoic acid)	20.	Eicosenoic acid	32.	Eicosadienoic acid
9.	Dihomo-gamma-linolenic acid (DGLA)	21.	Heneicosylic acid (heneicosanoic acid)	33.	Arachidonic acid (AA)
10.	Eicosatrienoic acid (ETE)	22.	Eicosapentaenoic acid (EPA, Timnodonic acid)	34.	Behenic acid (docosanoic acid)
11.	Erucic acid	23.	Docosadienoic acid	35.	Tricosylic acid (tricosanoic acid)
12.	Lignoceric acid (tetracosanoic acid)	24.	Docosahexaenoic acid (DHA, Cervonic acid)	36.	Nervonic acid

Hongdo and Hataedo Island (Table 1). Among them, the number of samples collected in summer, when *K. koreana* are most active, was largest with 43, followed by 33 in autumn, 16 in winter, and 7 in spring. While the number of samples in Hongdo Island was 69, the number of samples in Hataedo Island was 30, because ship was canceled several times due to influence of northwest wind, making it difficult to enter Hataedo Island.

2. Fatty Acid Analysis

Using fatty acid methyl ester (FAME) analysis, highly volatile fatty acids were methyl

Table 3. 36 oven conditions

	Rate ℃/min	Value ℃	Hold Time min	Run Time min
(Initial)		50	1	1
Ramp 1	25	130	0	4.2
Ramp 2	8	170	0	9.2
Ramp 3	2	215	10	41.7
Ramp 4	10	250	0	45.2

esterified with methanol to convert them into stronger volatile derivatives, and each component was separated by gas chromatography (GC). According to temporal and spatial differences of collected *K. koreana* content of a total of 36 fatty acids was analyzed by dividing them by season, region, and microhabitat (Table 2). Fatty acid analysis method followed Garces and Manuel (1983). Pretreatment method for samples is as follows. 1) Only fresh tissues of samples were removed and freeze-dried to prevent decomposition of sample; 2) samples were finely ground using a freeze-grinding machine; 3) crushed samples and standard (supelco 37 component FAME mix, Supelco, USA) were added to a 4 ml bottle with a Teflon cap; 4) samples were heated by mixing 2 ml of reagent methylation mixture [MeOH : Benzene : DMP (2, 2 − Dimethoxy - propane) : H_2SO_4 = 39 : 20 : 5 :2] and 1 ml of heptane at 80℃ for 2 hours; 5) extract supernatant formed by cooling at room temperature and analyze it with GC. GC conditions are as follows (Table 3). 1) GC: Agilent 7890A (Agilent, USA); 2) column: DB-23 (Agilent, 60mm*0.25mm*0.25um); 3) injector: 250℃; 4) detector: FID(280℃, H2 35, Air 350, He 35ml/min); 5) injection: 1uL (spilt ratio 10).

Ⅲ. Result and Discussion

1. Total Fatty Acid Composition of *K. koreana*

Since fatty acids in animals generally reflect fatty acid composition of ingested food sources, changes in feeding environment can be predicted by tracking composition of fatty acids of organisms (Shin and Kim, 2010). A total of 16 fatty acids were detected in tissues of *K. koreana* (Fig. 1), of which C16 : 0, C18 : 0, C18 : 1ω9, C18 : 2ω6, C18 : 3ω3, and C20 : 4ω6, C20 : 5ω3 are known to be commonly detected in mollusks or invertebrates including land snails (Ekin, 2015; Sinanoglou and Miniadis-Meimaroglou, 1998). C20 : 4ω6 (4.21 mg/g), essential fatty acid and prostaglandin precursors in organisms, was main components followed by C18 : 2ω6 (3.65 mg/g), C18 : 0 (3.42 mg/g) and C18 : 1ω9 (3.22 mg/g). Especially, *K. koreana* contained significant amount of essential fatty acid, C18 : 2ω6 which plays a crucial role in synthesis of other fatty acids. On the other hand, only a small amount of C14 : 0 (0.09 mg/g) and C17 : 0 (0.60 mg/g) were detected in short chain fatty acids mainly assimilated from microorganisms. Quantity of fatty acids is influenced by environmental, nutritional, and physiological factors, and notably, short-chain

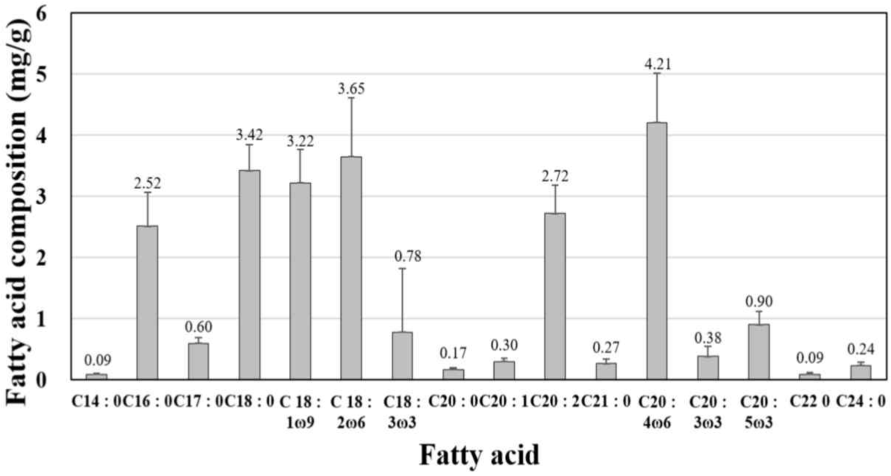

Figure 1. Average and composition of 16 fatty acids of *K. koreana*

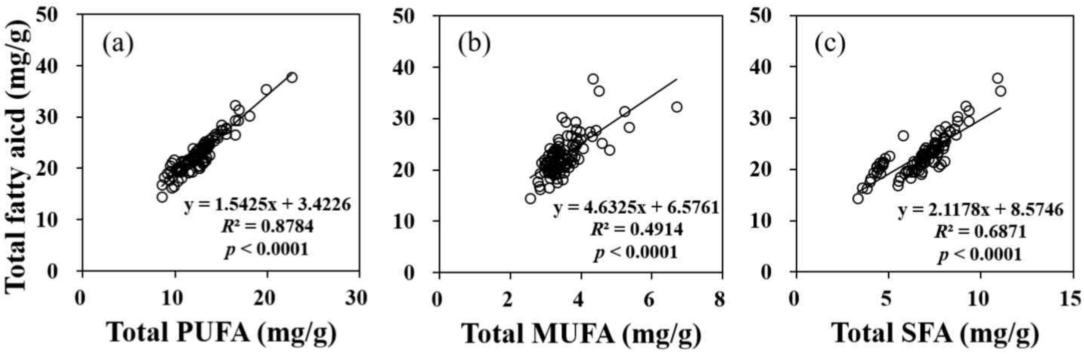

Figure 2. Pearson correlation analysis of (a) PUFA, (b) MUFA and (c) SFA relative to the total amount of fatty acids

fatty acids in snails inhabiting forests are lower compared to urban areas (Ekin, 2015). This indicates that habitat environment of *K. koreana* is not polluted and remains in a pristine state.

2. Comparative of PUFAs, MUFAs and SFAs

Same number of unsaturated fatty acids and saturated fatty acids were detected as 8 each (Table 4). However, in the case of detection amount (mg/g), relative ratio of unsaturated fatty acids (68.6%) was more than twice that of saturated fatty acids (31.4%). Among the fatty acids detected, ratio of particularly highly polyunsaturated fatty acids (PUFAs) was 53.6% on average, highest compared to 15.0% of monounsaturated fatty acids (MUFAs) and 31.4% of saturated fatty acids (SFAs). This is consistent with findings of fatty acid studies in herbivorous snails such as *A. escheriana* and *A. Guttata*, which also showed a significantly higher proportion of PUFAs (Ekin, 2015). Additionally, total amount of fatty acids showed strong correlation with changes in PUFAs (R^2=0.88, p < 0.0001), followed by SFAs (R^2=0.69, p < 0.0001) and MUFAs (R^2=0.49, p < 0.0001) (Fig. 2). Given that plant-based diets contain significantly more PUFAs compared to

Table 4. The composition of average fatty acids by season, region, and microhabitat of *K. koreana*

No.	fatty acid		season				region		microhabitat		average
			spring	summer	autumn	winter	Hongdo	Hataedo	grassy	calcareous	
1.	Myristic acid[1] (tetradecanoic acid)	C14: 0	-	-	0.09±0.02	0.09±0.01	0.09±0.02	0.09±0.01	0.08±0.01	0.10±0.01	0.09±0.01
2.	Palmitic acid[1] (hexadecanoic acid)	C16: 0	-	2.59±0.51	2.46±0.65	2.30±0.17	2.49±0.58	2.64±0.46	2.58±0.63	2.52±0.55	2.52±0.56
3.	Margaric acid[1] (heptadecanoic acid)	C17: 0	0.72±0.10	0.59±0.08	0.58±0.10	0.61±0.09	0.58±0.09	0.64±0.10	0.59±0.07	0.60±0.10	0.60±0.09
4.	Stearic acid[1] (octadecanoic acid)	C18: 0	3.41±0.36	3.52±0.37	3.43±0.49	3.20±0.40	3.46±0.41	3.34±0.45	3.42±0.40	3.4±0.46	3.42±0.43
5.	Oleic acid[2]	C18: 1ω9	3.34±0.33	3.27±0.66	3.14±0.46	3.19±0.44	3.13±0.47	3.41±0.65	3.40±0.73	3.11±0.38	3.22±0.55
6.	Linoleic acid[3]	C18: 2ω6	3.61±0.70	3.86±1.00	3.57±1.12	3.31±0.51	3.63±0.99	3.68±0.91	4.02±1.21	3.46±0.8	3.65±0.96
7.	Alpha-linolenic[3] acid (ALA)	C18: 3ω3	0.29±0.06	0.89±0.89	1.01±1.51	0.36±0.09	0.88±1.21	0.58±0.52	0.98±1.03	0.73±1.12	0.78±1.04
8.	Arachidic acid[1] (icosanoic acid)	C20: 0	0.19±0.02	0.17±0.03	0.17±0.04	0.16±0.02	0.17±0.03	0.17±0.02	0.17±0.03	0.17±0.03	0.17±0.03
9.	Eicosenoic acid[2]	C20: 1	0.28±0.04	0.31±0.07	0.30±0.05	0.31±0.04	0.31±0.06	0.30±0.04	0.31±0.07	0.30±0.05	0.30±0.06
10.	Eicosadienoic acid[3]	C20: 2	3.00±0.31	2.80±0.47	2.45±0.35	2.87±0.46	2.68±0.41	2.81±0.56	2.65±0.45	2.72±0.46	2.72±0.46
11.	Heneicosylic acid[1] (heneicosanoic acid)	C21: 0	0.20±0.03	0.30±0.06	0.26±0.07	0.23±0.06	0.28±0.07	0.24±0.06	0.29±0.07	0.25±0.07	0.27±0.07
12.	Arachidonic[3] acid (AA)	C20: 4ω6	4.84±0.39	4.23±0.85	3.78±0.66	4.61±0.65	4.17±0.77	4.29±0.88	3.91±0.79	4.25±0.74	4.21±0.80
13.	Eicosatrienoic[3] acid (ETE)	C20: 3ω3	0.37±0.09	0.40±0.14	0.39±0.23	0.34±0.07	0.39±0.19	0.37±0.07	0.38±0.13	0.39±0.18	0.38±0.16
14.	Eicosapentaenoic acid[3] (EPA, Timnodonic acid)	C20: 5ω3	1.09±0.29	0.90±0.19	0.87±0.23	0.91±0.18	0.90±0.21	0.91±0.22	0.86±0.19	0.93±0.23	0.90±0.21
15.	Behenic acid[1] (docosanoic acid)	C22: 0	-	0.11±0.05	0.08±0.02	0.09±0.01	0.09±0.03	0.09±0.01	0.09±0.04	0.09±0.03	0.09±0.03
16.	Lignoceric acid [1] (tetracosanoic acid)	C24: 0	0.27±0.03	0.24±0.06	0.22±0.05	0.24±0.04	0.24±0.06	0.24±0.04	0.23±0.06	0.24±0.05	0.24±0.05
∑SFA/ ∑SFA(%)			4.80 (22.1)	7.53 (31.1)	7.28 (32.0)	6.92 (30.3)	7.39 (31.5)	7.45 (31.3)	7.46 (31.1)	7.37 (31.7)	7.40 (31.4)
∑MUFA/ ∑MUFA (%)			3.62 (16.8)	3.58 (14.8)	3.44 (15.1)	3.50 (15.4)	3.44 (14.6)	3.71 (15.6)	3.71 (15.5)	3.41 (14.6)	3.52 (15.0)
∑PUFA/ ∑PUFA (%)			13.20 (61.1)	13.08 (54.1)	12.06 (52.9)	12.40 (54.3)	12.65 (53.9)	12.64 (53.1)	12.80 (53.4)	12.48 (53.7)	12.65 (53.6)

Values are means ± Standard Deviation for samples
[1]SFA: Saturated fatty acid, [2]MUFA: Monounsaturated fatty acid, [3]PUFA: Polyunsaturated fatty acid

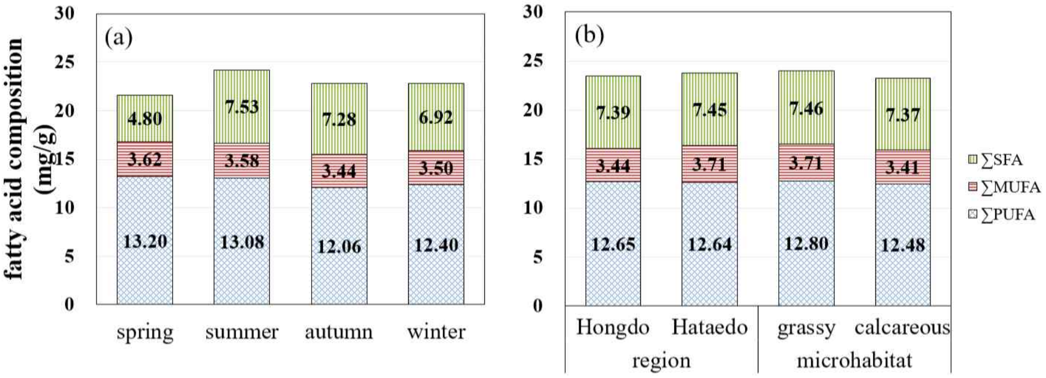

Figure 3. Average fatty acid detection of *K. koreana* by (a) season, (b) region and microhabitat

carnivorous diets (Ekin, 2015). These results suggest that PUFAs is the effective indicator for habitat quality change of *K. koreana*.

Detection of seasonal PUFAs was slightly higher in spring (13.20 mg/g) and summer (13.08 mg/g) than in autumn (12.06 mg/g) and winter (12.40 mg/g) (Fig. 3). Because aging or decay of plants results in a decrease in PUFAs content (Kis et al., 1998), fatty acid composition of land snail in habitats can vary with seasons (Wacker, 2005). It is considered that change in plant freshness according to these seasonal effects influenced seasonal fatty acid composition of the *K. koreana*. Detection amount of PUFAs in grassy habitat (12.80 mg/g) was relatively higher than that of calcareous habitat (12.48 mg/g), but there was no obvious difference. This is presumed to be related to abundance of herbaceous plants surrounding stone wall. As a result of analyzing fatty acids according to geographical isolation, PUFAs detected in individuals collected in Hongdo (12.65 mg/g) and Hataedo (12.64 mg/g) were very similar. Accordingly, it is considered that quality of food sources of Hongdo and Hataedo Island is very similar. However, just as the detection of PUFAs decreased during the winter season, it can be predicted that if the vegetation in the microhabitat or region decreases, PUFAs will also decrease. Until further research is conducted on the correlation between the changes in PUFAs and population size of *K. koreana*, the PUFAs detected in this study are expected to serve as a standard for habitat quality management.

3. Conservation of *K. koreana*

Land snails have low mobility, so allopatric speciation can occur relatively easily. In particular, many cases of adaptive radiation are known on Oceanic islands such as the Bermuda Islands and the Ogasawara Islands (Chiba, 1999; Chiba and Cowie, 2016; Outerbridge and Sarkis, 2018), but many species of native snails are already extinct or endangered. In addition, most of native genus *Poecilozonites* in the Bermuda Islands have already been extinct, but some species of natural population have recently been rediscovered and various restoration projects are underway (Outerbridge and Sarkis, 2018; Copeland and Hesselberg, 2021). *K. koreana* Pfeiffer (1850) is a representative species of adaptive radiation found only on some islands in Sinan-gun, Jeollanam-do, South Korea, and is protected by the Ministry of Environment as

Class II endangered species. Therefore, there is a need to develop quantitative indicators for assessing habitat quality of *K. koreana*.

Quality of food sources has a major impact on growth and reproduction of animal communities (Mike et al., 2004; Pernet et al., 2005). In particular, this study confirms importance of plant-based food for *K. koreana*, and it is predicted that quality of plant-based food can be tracked through changes in PUFAs content in fatty acids. PUFAs can be modified to synthesize prostaglandin in the body (Stanley-Samuelson, 1987), which directly stimulates snail egg production (Kunigelis and Saleuddin, 1986). In addition, snails fed with insufficient PUFAs reduce mating activity (Ekin, 2015), leading to decreased reproduction. Accordingly, it is considered that PUFAs content can be used as a habitat quality indicator for habitat conservation policy of *K. koreana.* For the conservation of land snail habitats, maintaining stable vegetation is paramount, and person-centered physicochemical herbicidal activities within these habitats should be prohibited. In the future, it is necessary to identify key plant food sources through metabarcoding studies utilizing *K. koreana* feces, allowing these to be collectively protected within *K. koreana* habitats as part of habitat management.

IV. Conclusion

Fatty acid analysis of designated endangered species *K. koreana* (Mollusca: Gastropoda) by the Ministry of Environment resulted in following conclusions. In fatty acid analysis of *K. koreana*, a total of 16 different types were detected, among which C20 : 4ω6, C18 : 2ω6, C18 : 0, and C18 : 1ω9 were identified as major fatty acids. Among them, PUFAs accounted for more than half of total and total amount of fatty acids showed strongest correlation with changes in PUFAs. Food sources status according to season, microhabitat, and region was effectively reflected in the changes in the fatty acid composition of *K. koreana*. Therefore, PUFAs is effective physiological indicator for habitat quality change of *K. koreana* and it is anticipated that this can be utilized in habitat conservation policy. Ensuring the preservation of steady vegetation stands as crucial for safeguarding *K. koreana* habitats, thus warranting the prohibition of human-induced physicochemical herbicidal practices within these environments for conservation purposes.

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