

Determination of feed value of chemically treated sun-dried grape pomace

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ABSTRACT

This study aimed to elucidate the effects of various chemical treatments on the *in vitro* dry matter digestibility (IVDMD) and concomitant feed value of dried grape pomace (DGP). Sun-dried grape pomace, derived from grapes processed in Denizli, Türkiye province was used in the experiment. One control group and three treatment groups were established, each with eight replicates. The first, second and third experimental groups were treated with 1.5% ammonia (NH₃), 3% urea (CH₄N₂O) and 3% sodium hydroxide (3%) (NaOH), respectively. The dry matter content was set at 50% for all groups. The control group received no additional chemicals. All groups were kept in airtight, closed plastic bags at room temperature for one week. Adding NH₃ and CH₄N₂O significantly improved crude protein levels (24.45 and 19.84%, respectively) and *in vitro* protein degradability (59.84 and 65.69%, respectively) in the experimental groups (p<0.001). Treatment with CH₄N₂O did not exert the same deleterious effect on the ether extract concentration of desiccated grape pomace (p<0.004) as treatment with NaOH. Moreover, relative feed value declined significantly more after NaOH treatment compared to treatments containing nitrogen (p<0.001). Additionally, NaOH treatment yielded the lowest *in vitro* protein degradability of DGP at 40.32% (p<0.001). With an *in vitro* dry matter degradability of 47.18%, the findings indicate that among the chemical treatments investigated, application of NH₃ resulted in the lowest degradability value (p<0.001). Consequently, it can be inferred that the feed value and digestibility of desiccated grape pomace are highly influenced by the nitrogen concentration or basic nature of the supplemental chemical compounds.

INTRODUCTION

The utilization of nutritionally valuable by-products in animal nutrition is common in the agriculture sector. Grape pomace is, however, one of these underutilized agricultural by-products. Although grape production areas in Türkiye have decreased since 2013 (Sümbül and Yıldız, 2022), FAO statistics put it 7th in the world with 390 thousand hectares in 2021 (Faostat, 2023). Türkiye produced 4 million 165 thousand tons of grapes in 2022 (2 million 99 thousand for table, 1 million 681 thousand for drying, and 383 thousand for wine). This production constitutes 15.5% of the country's total output in the fruits, beverages, and spices category (TUIK, 2023). The waste product from pressing grapes for wine or juice is called grape pomace. Varış et al. (2000) state that approximately 15-25% of grapes used in wine production are left as grape pomace. Nerantzis and Tataridis (2006) describe grape pomace' composition as 24.9% stem, 22.5% seed, and 42.5% peels. The chemical composition of pomace varies depending on the grape variety, climate conditions, and winemaking techniques (Deng et al., 2011). Due to its high moisture content, grape pomace is often recommended to be processed by drying or ensiling for use in animal feed (Özdüven et al., 2005).

Bahrami et al. (2010) found that incorporating 10% grape pomace in the diet did not adversely affect lamb growth. The addition of 20% grape pomace to heifer rations had no neg-

ative effect on fattening performance or feed intake (Voicu et al., 2014). Zhen-Zhen et al. (2015) noted that incorporating 8-16% grape pomace in lamb diets improves feed conversion ratio, daily live weight gain, and several carcass characteristics. The present experiments evince that grape pomace can serve as a partial replacement for roughage, and that augmenting its feed value may engender improved performance. Chemical analyses have demonstrated that the crude protein (CP) content of grape pomace ranges from 6.6% (Besharati and Taghizadeh, 2010) to 17.27% (Aghsaghali et al., 2011). Similar variations in acid detergent fiber (ADF) and neutral detergent fiber (NDF) content have been reported. For instance, Besharati and Taghizadeh (2010) reported pomace's ADF and NDF levels as 18.4% and 18.7%, respectively, while Atalay (2020) reported them as 53.88% and 58.8%. According to Sarıçiçek and Kılıç (2002), grape pomace had a 48-hour dry matter (DM) degradability of 18.57%, an organic matter degradability of 16.19%, a low crude protein degradability of 19.80%, and a high pepsin solubility of 42.80%. *In vitro* studies conducted by Kılıç and Abdiwali (2016) revealed that DGP exhibited higher true digestibility, DM digestibility, and relative feed value (RFV), along with increased dry matter intake (DMI), compared to grape seeds.

Recent investigations have evinced that the application of different chemical compounds to low-quality or alternative

forages can decrease ADF and NDF levels, thereby enhancing the digestibility of DM and organic matter (Rezaei et al., 2023; Uzatici et al., 2022; Maduro Dias et al., 2021). Rezaei et al. (2023) found that treatment of cumin straw with $\text{CH}_4\text{N}_2\text{O}$ led to an increase in its NDF level. Uzatici et al. (2022) demonstrated that applying 1-3% sodium hydroxide (NaOH) to cane (*Phragmites australis*) reduced its NDF and ADF content while enhancing the digestibility of total DM (1-3%) and organic matter (2 and 3%). Rezaei et al. (2023) also observed that hydrogen peroxide treatment of cumin straw increased its total digestible nutrients (TDN) but decreased both the amount of ADF and DM digestibility. Maduro Dias et al. (2021) reported that treating ginger lily components with 8% NaOH not only decreased the plant's ADF and NDF levels but also increased its *in vitro* digestibility of dry matter (IVDMD) and organic matter. Similar results were observed in the *Arundo donax* plant treated with 8% NaOH (Teixeira et al., 2021).

The NaOH utilized in the present study is classified as an acidity regulator for cats, dogs, and ornamental fish according to Annex I of the EU Feed Additives Regulation published on January 21, 2013 (Code 1j524). However, NaOH can additionally be employed in product processing as part of its designated application under the 'special use' categorization (EC 2003). Conversely, $\text{CH}_4\text{N}_2\text{O}$ is also permitted for use in ruminants (EC 2012). It was observed that there exists no regulation governing the use of liquid NH_3 in animal feed. The purpose of this investigation is to explore whether grape pomace, a by-product of the grape industry, can be utilized as a roughage supplement in animal husbandry. To this end, an assessment was conducted on the nutritional value, *in vitro* protein degradability, and IVDMD of DGP in response to interventions involving NH_3 , $\text{CH}_4\text{N}_2\text{O}$, and NaOH, all of which constitute economically viable additives for producers.

MATERIALS and METHODS

DGP from the province of Denizli-Türkiye was used in the study. Three experimental groups and one control group were established, each consisting of eight replicates. Solutions of 1.5% ammonia (DGP- NH_3), 3% urea (DGP- $\text{CH}_4\text{N}_2\text{O}$), and 3% sodium hydroxide (DGP-NaOH) were prepared in distilled water and applied (by hand mixing) to the first, second, and third experimental groups, respectively. The DM content was standardized to 50% for all groups. The study methodology employed herein adheres to the approaches delineated by Martens et al. (2022) and Uzatici et al. (2022) for determining the requisite quantities of NaOH and $\text{CH}_4\text{N}_2\text{O}$, as well as for standardizing the treatments at 50% DM. The treatment level was established at 1.5%, thereby ensuring that the application of liquid NH_3 in this investigation did not perturb the 50% DM standardization. The control group received no chemical additives, and distilled water was used to adjust moisture levels. All the study groups were stored in airtight plastic bags at room temperature for one week (Martens et al., 2022; Teixeira et al., 2021).

Determination of nutritional content of groups

The levels of DM, crude ash (CA), CP, and ether extract (EE) in the samples were determined using the techniques

outlined in AOAC (2000). The quantification of crude fiber (CF), NDF, ADF, and acid detergent lignin (ADL) was performed using an ANKOM A2000 Fiber Analyzer (ANKOM Technology, NY, USA) (Ahsan, 2023).

Determination of roughage qualities and relative feed value

To calculate the forage quality (including total carbohydrate, non-structural carbohydrate, cellulose, hemicellulose, net energy lactation (NEL), and total digestible nutrient (TDN) content and (RFV) of the control and experimental groups, the following formulas were used (Ahsan, 2023; Horrocks and Vallentine, 1999).

- Total carbohydrates (in dry matter) = $\text{DM}\% - (\text{CP}\% + \text{CA}\% + \text{EE}\%)$
- Non-structural carbohydrates (% DM basis) = $100 - (\text{NDF}\% + \text{CP}\% + \text{CA}\% + \text{EE}\%)$
- Cellulose (% DM basis) = $\text{ADF}\% - \text{ADL}\%$
- Hemicellulose (% DM basis) = $\text{NDF}\% - \text{ADF}\%$
- NEL (Mcal/kg) = $[1.044 - (0.0119 \times \text{ADF}\%)] \times 2.205$
- TDN (% DM basis) = $(-1.291 \times \text{ADF}\%) + 101.35$
- Digestible dry matter-DDM (% DM basis) = $88.9 - (0.779 \times \text{ADF}\%)$
- Dry matter intake-DMI (% DM basis) = $120 \div \text{NDF}\%$
- Relative feed value-RFV (% DM basis) = $\text{DDM}\% \times \text{DMI}\% \times 0.775$

RFV is a key metric used in the evaluation and marketing of roughage. According to Kılıç and Addi Abdiwali (2016), RFV values below 75 indicate poor quality, 75-86 signify 4th quality, 87-102 3rd, 103-124 2nd, 125-151 as good, and values greater than 151 are considered 1st quality.

Determination of *in vitro* rumen digestibility and crude protein degradability of groups

The IVDMD and organic matter digestibility (IVOMD) were determined for each group using the ANKOM Daisy II incubator. For this purpose, duplicate samples were weighed and placed into filter bags (25 mm pore size propylene polyester) compatible with the device. These filter bags were then placed in the rumen fluid incubator bottle. The ruminal contents utilized were obtained by combining those from three distinct bovine subjects (13-month-old Holstein steers) that had been slaughtered subsequent to receiving a diet consisting of barley and corn silage. The fluid was transported to the laboratory within 20 minutes, using a thermos maintained at a constant temperature of 39°C. The filter bags were subsequently subjected to a 48-hour incubation period in an ANKOM Daisy II incubator containing ruminal content, buffer A, and buffer B. The quantities of each component (KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{CH}_4\text{N}_2\text{O}$) utilized in the preparation of the Buffer A solution were as specified in the prescribed methodology. The constituents of the buffer B solution were Na_2CO_3 and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$. Anaerobic condi-

tions were maintained throughout the procedure by means of a continuous carbon dioxide gas flow. Finally, the values for IVDMD and IVOMD were calculated based on the process (Ahsan, 2023).

Statistical analysis

For statistical analysis, the software SPSS 18.0 (IBM Corp., Armonk, NY, US) was employed. The Kolmogorov-Smirnov test was utilized to assess whether the data followed a normal distribution. One-way analysis of variance (ANOVA) was performed to compare the differences in values across the groups. Duncan's multiple range test was used to determine the significance of differences in mean values between groups. Results are presented as marginal means and their associated standard errors. A p-value of less than 0.05 was considered statistically significant (Dawson and Trapp, 2001).

RESULTS

The nutrient content, and levels of fiber fractions (NDF, ADF, and ADL) of grape pomace treated with various chemicals are depicted in Table 1. Compared to the control and NaOH treatment groups, the application of NH_3 and $\text{CH}_4\text{N}_2\text{O}$ substantially increased CP levels (24.45 and 19.84%, respectively) ($p < 0.001$), with improvements ranged from 61-90%. Regarding EE content, the two N-containing groups (DGP- $\text{CH}_4\text{N}_2\text{O}$ and DGP- NH_3) exhibited similarity. The difference in EE content between these groups and the control

and DGP-NaOH groups did not reach statistical significance. Remarkably, all experimental groups exhibited significantly higher ADF, NDF, and ADL levels when compared with the control group ($p < 0.035$). Moreover, the DGP- $\text{CH}_4\text{N}_2\text{O}$ group demonstrated the highest CA content in comparison to the control and other experimental groups ($p < 0.001$).

The effect of chemical treatments on total carbohydrates (CHO), non-fibrous carbohydrates (NFC), cellulose and hemicellulose levels in grape pomace is shown in Table 2. In the study, no alteration was seen in calculated structural carbohydrate levels (cellulose and hemicellulose). Table 3 presents the feed value and *in vitro* digestibility of chemically treated grape pomace. Notably, all chemically treated experimental groups exhibited considerably lower values for digestible DDM, DMI, RFV, NEL, and TDN compared to the control group ($p < 0.001$). Interestingly, no significant differences were found between the experimental groups treated with N-containing chemicals (DGP- $\text{CH}_4\text{N}_2\text{O}$ and DGP- NH_3). The IVPD values for the two N-containing experimental groups (DGP- $\text{CH}_4\text{N}_2\text{O}$ and DGP- NH_3) were 65.69% and 59.84%, respectively. Although a significant difference existed between these values, they were naturally significantly higher ($p < 0.001$) than that of the DGP-NaOH group (40.32%). Conversely, the IVDMD and IVOMD values of both N-containing experimental groups were significantly reduced compared to the control group ($p < 0.001$).

Table 1. Nutrient content and NDF, ADF, and ADL levels of grape pomace treated with chemicals (% DM basis).

Nutrients	Control	Experimental Groups			p
		DGP- NH_3	DGP- $\text{CH}_4\text{N}_2\text{O}$	DGP-NaOH	
DM	94.04 ± 0.04 c	94.94 ± 0.24 b	95.90 ± 0.03 a	94.90 ± 0.02b	0.001
CF	31.31 ± 0.23 b	33.55 ± 0.36 a	32.19 ± 0.40 b	30.19 ± 0.37 c	0.001
EE	3.78 ± 0.15 bc	4.35 ± 0.23 a	4.06 ± 0.11 ab	3.50 ± 0.13 c	0.004
CP	12.86 ± 0.15 c	24.45 ± 0.22 a	19.84 ± 0.47 b	12.32 ± 0.18 c	0.001
CA	6.14 ± 0.07 b	5.88 ± 0.05 b	9.97 ± 0.30 a	6.52 ± 0.38 b	0.001
ADF	19.21 ± 0.33 b	20.53 ± 0.39 a	21.11 ± 0.43 a	21.61 ± 0.41 a	0.001
ADL	13.07 ± 0.31 b	14.53 ± 0.40 a	14.72 ± 0.58 a	14.75 ± 0.48 a	0.035
NDF	23.08 ± 0.36 b	24.51 ± 0.40 a	24.88 ± 0.41 a	25.73 ± 0.74 a	0.005

DM: Dry matter, CF: Crude fiber, EE: Ether extract, CP: Crude protein, CA: Crude ash, ADF: Ash-free acid detergent fiber, ADL: Acid detergent lignin, NDF: Ash-free neutral detergent fiber after amylase treatment.

Table 2. The effect of chemical treatments on total CHO, NFC, cellulose, and hemicellulose levels in grape pomace (% DM basis).

Nutrients	Control	Experimental Groups			p
		DGP- NH_3	DGP- $\text{CH}_4\text{N}_2\text{O}$	DGP-NaOH	
Total CHO	71.25 ± 0.27 b	60.25 ± 0.37 d	62.02 ± 0.56 c	72.55 ± 0.46 a	0.001
NFC	54.12 ± 0.44 a	40.79 ± 0.41 c	41.22 ± 0.67 c	51.91 ± 0.75 b	0.001
Hemicellulose	3.86 ± 0.07	3.98 ± 0.09	3.76 ± 0.25	4.11 ± 0.41	0.788
Cellulose	6.13 ± 0.07	6.00 ± 0.18	6.39 ± 0.34	6.86 ± 0.39	0.155

Total CHO: Total carbohydrates, NFC: Non-fibrous carbohydrates.

Table 3. Feed value and *in vitro* digestibility of grape pomace treated with chemicals (% DM basis).

	Experimental Groups				p
	Control	DGP-NH ₃	DGP-CH ₄ N ₂ O	DGP-NaOH	
<i>Feed value</i>					
DDM	73.93 ± 0.26 a	72.77 ± 0.32 b	72.44 ± 0.33 b	70.39 ± 0.35 c	0.001
DMI	5.21 ± 0.08 a	4.91 ± 0.08 b	4.84 ± 0.08 b	4.12 ± 0.06 c	0.001
RFV	299.25 ± 5.80 a	277.56 ± 5.98 b	272.25 ± 6.13 b	225.40 ± 4.59 c	0.001
NEL	1.79 ± 0.01 a	1.74 ± 0.01 b	1.74 ± 0.01 b	1.67 ± 0.01 c	0.001
TDN	76.54 ± 0.43 a	74.11 ± 0.87 b	74.08 ± 0.55 b	70.69 ± 0.59 c	0.001
<i>In vitro</i> digestibility					
IVPD	47.63 ± 1.60 c	59.84 ± 1.34 b	65.69 ± 0.98 a	40.32 ± 1.51 d	0.001
IVDMD	59.43 ± 0.60 a	47.18 ± 0.69 c	54.39 ± 0.76 b	56.94 ± 1.59 ab	0.001
IVOMD	58.45 ± 0.61a	46.22 ± 0.73 b	47.91 ± 1.02 b	55.39 ± 1.69 a	0.001

DDM: Digestible dry matter (% DM basis), DMI: Dry matter intake (% body weight), RFV: Relative feed value, NEL: Net energy for lactation (Mcal/kg), TDN: Total digestible nutrients (% DM basis), IVPD: In vitro protein degradability (% DM basis), IVDMD: In vitro true dry matter digestibility (% DM basis), IVOMD: In vitro true organic matter digestibility (% DM basis).

DISCUSSION

The CP levels observed in the control and DGP-NaOH groups, 12.86% and 12.32% respectively, were comparable to the 12.5% level reported for dried grape pomace (DGP) by Kılıç and Abdiwali (2016). Several studies (Pop et al., 2015; Basalan et al., 2011; Baumgartel et al., 2007) indicate that there may be a difference in CP levels between 2.1 and 6.2% in favor of red grape pomace. Furthermore, the findings of Hanusovsky et al. (2020) evinced that the crude protein content of pomace derived from the same grape variety can exhibit a disparity of approximately 2.7% when cultivated in distinct geographical locales. The soil and climate where the grape grow will influence the nutritional level, although variations in processing methods could also play a role. The CP levels were found to be elevated in DGP treated with NH₃ or CH₄N₂O. It is plausible that the high nitrogen content of these two chemical compounds may be responsible for the observed increase in CP levels. The EE level obtained by NaOH treatment of DGP is congruent with the findings of Sarıççek and Kılıç (2002) (3.67%). Notably, the EE ratio of the DGP-NH₃ group (4.35%) is substantially higher than that of the control and NaOH groups. This phenomenon could be attributable to either the interaction between NH₃ and oil or the hydrolysis of lipids facilitated by NaOH. Basalan et al. (2011) reported that the EE content in grape pomace seed, membrane plus soft tissue, and stem is 6.2%, 4.6%, and 1.2%, respectively.

Numerous studies have reported that the CF content of pomace is influenced by factors such as the grape variety, the proportion of stems present in the pomace, and the region where the grapes are cultivated (Hanusovsky et al., 2020; Pop et al., 2015; Baumgartel et al., 2007). According to Baumgartel et al. (2007) and Pop et al. (2015), red grape pomace exhibited a CF content of 31.2%, stemless red grape pomace had 31.31%, and stalked white grape pomace contained 32.28%. These values are consistent with those observed for the untreated

pomace (control) and DGP-CH₄N₂O groups in the present study. However, according to reports, the CF content of grape pomace from Slovakia's Zwegelt and Austria's Green Veltliner is 23.3% and 12%, respectively (Hanusovsky et al., 2020). Sarıççek and Kılıç (2002) reported 18.6% CA in grape pomace. The grape species could be the cause of the high CA level. The results of the study are consistent with the CA values of white-stemmed and red-stemmed grape pomace (6.46% and 6.51%, respectively) in the control, DGP-NH₃, and DGP-NaOH groups. The high CA of the DGP-CH₄N₂O group in the study may be due to the purity level of the CH₄N₂O used or the seed content of the pomace mixture. Özcan et al. (2017) reported that the mineral content of grape seed is higher compared to the rest of the grape. Similar to those measured from Green Veltliner grape pomace produced in Austria (Hanusovsky et al., 2020), the values of ADF, NDF, ADL in the experimental groups of this study are in agreement with those reported.

There is a lack of study about the effects of chemical treatment on the structural carbohydrates of DGP. However, certain studies have demonstrated that the ADF, NDF, and ADL levels of pomace are influenced by the country where the grape is produced (the soil or climate in which it grows) (Hanusovsky et al., 2020), as well as its variety (Kılıç and Abdiwali, 2016; Winkler et al., 2015; Baumgartel et al., 2007). Hanusovsky et al. (2020) reported that structural carbohydrates in a single grape variety grown in Slovakia can exhibit variations of 4.9 to 5.9%. The present study's ADF, NDF, and ADL values for all chemical groups were lower compared to the results of earlier studies (Kılıç and Abdiwali, 2016; Winkler et al., 2015; Baumgartel et al., 2007) on grape pomace. The literature indicates that the ADF content of white grape pomace was 43.7% (Winkler et al., 2015), while the NDF content was 50.7% (Baumgartel et al., 2007). Regarding DGP, the ADL was reported as 32.4% (Kılıç and Abdiwali, 2016).

Chemical treatments did not significantly alter the amounts of cellulose and hemicellulose in the groups. The cellulose content observed in DGP aligns with the findings of Kılıç and Abdiwali (2016). Similarly, the hemicellulose level mirrors the results reported for Austrian Pinot Blanc grape pomace by Honisovsky et al. (2020). The NFC values of the control and DGP-NaOH groups resemble those of Austrian Green Veltliner grape (54%). Conversely, the NFC values of the DGP-NH₃ and DGP-CH₄N₂O groups more closely match the values of Pinot Blanc grape pomace (41.5%) (Hanusovsky et al., 2020). The reduction in pomace's NFC value induced by both N-containing chemicals may correlate with the concurrent increase in its nitrogen content.

Kılıç and Abdiwali (2016) reported that DGP had DDM, DMI, and RFV of 62.61, 2.74, and 133.06%, respectively. These values are lower than those observed in our trial and control groups in the present study. Within the present study, the DGP-NaOH group exhibited the lowest relative feed value (RFV) at 225.40. However, it should be noted that Linn and Martin (1999) have posited that an RFV score exceeding 151 is indicative of roughage meeting the highest quality classification criteria. Investigations examining the *in vitro* dry matter digestibility (IVDMD) of grape pomace are relatively scarce. Nonetheless, the IVDMD levels observed in the control and DGP-NaOH groups were congruent with the report by Kılıç and Abdiwali (2016) (62.61%). This variation may be attributed to differences in grape species and the proportion of stems to seeds. Çakmakçı and Barut (1997) noted that NaOH treatment of low-nutritional-value forages solubilized some hemicellulose without affecting the cellulose concentration. Various studies have shown that NaOH treatment of alternative forages enhances their IVDMD (Uzatici et al., 2022; Maduro et al., 2021; Teixeira et al., 2021). The IVDMD results for DGP-NaOH are consistent with literatures. However, the IVDMD of pomace was significantly reduced following NH₃ treatment compared to the control and other trial groups. The reduction in digestibility might be attributed to the low hemicellulose levels (3-4%) in the DGP used in the present study. Currently, literature lacks data on protein degradability for DGP. In the present study, the IVPD in the DGP-NH₃ and DGP-CH₄N₂O groups were significantly higher compared to the DGP-NaOH and control groups. Nevertheless, it is evident that both nitrogen (N)-containing chemicals, NH₃ and CH₄N₂O significantly enhance the CP level in pomace.

CONCLUSION

The nutritional composition of DGP is significantly affected by chemical treatments. N-containing chemicals such as CH₄N₂O and NH₃ were found to increase DGP's IVPD and CP levels. Conversely, IVDMD decreased with NH₃ treatment, while NaOH treatment led to its increase. All groups exhibited RFV values meeting the criteria for top-level roughages in the study. However, NaOH treatment resulted in a lower RFV value for DGP compared to other chemical treatments. Further studies on the feed value and IVDMD of DGP may benefit from maintaining lower moisture content, thereby prolonging the incubation period, which holds potential for enhancing the literature through extended chemical application periods.

DECLARATIONS

Ethics Approval

The research is not an animal experiment, ethics committee authorisation is not required.

Conflict of Interest

The authors have no conflict of interest with any person, institution or organisation.

Consent for Publication

Publication is appropriate

Author contribution

Idea, concept and design: KEB

Data collection and analysis: KEB, DMK, EÇU, AN

Drafting of the manuscript: KEB

Critical review: KEB, MNO, FKO

Data Availability

The data is available from the corresponding author on reasonable request.

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