Research Article

Quality of Wafer Complete Feed for Ruminant with Virgin Coconut Oil (VCO) Supplementation by Non-Heating Method

Desy Cahya Widianingrum[1*], Himmatul Khasanah[1], Melinda Erdya Krismaputri[1], Susan Barbara Patricia Sembiring Meliala[2]

[1]Department of Animal Husbandry, Faculty of Agriculture, University of Jember, Jember, 68121, Indonesia; [2]Department of Agricultural Science, Faculty of Agriculture, University of Jember, Jember, 68121, Indonesia.

Abstract | This study aims to determine the quality of wafer with supplementation of protected Virgin Coconut Oil (VCO) made by the non-heating method. The ration of the wafer consisted of 30% rice straw, 30% concentrate, 30% *indigofera*, 5% molasses, and 5% VCO. The group of treatments were wafer supplemented with VCO (P0); wafer supplemented with VCO saponification (P1); wafer supplemented with VCO protected by formaldehyde (P2); wafer without VCO (P3); and wafer supplemented with VCO mixed on *indigofera* (P4). Each treatments were using six replications and tested for their physical quality, rancidity, and rumen fermentation characteristics and *in vitro* digestibility. The data obtained were analyzed using Analysis of Variance (ANOVA) and further tested with an Honestly Significant Difference (Hsd). The highest significant difference in physical quality of wafer ($P<0.05$) were found among treatments. The P4 group had the highest density (0.230 ± 0.16 g/cm^3), while the P1 group had the highest absorption (77.60%). The P0 group had the highest thickness swelling (12.95 ± 0.04 %), while both of P1 and P3 had the highest texture hardness (52.20 ± 0.14 and 52.19 ± 0.14 mm/10^2) respectively. The P3 group had the lowest rancidity (2.04 ± 0.01 mmol/kg). The advantage of VCO protection with tanin (mix on indigofera) has the lower rancidity value (2.14 ± 0.09 mmol/kg) compared to wafers with VCO supplementation. In the addition of VCO without treatment (P0) increase the total digestibility of dry matter (78.89 ± 0.06%) and organic matter (78.49 ± 0.16%). Our results showed that VCO treatments have not negative effect on the rumen environment (pH and NH_3 level), both protective and non-protective VCO, in the *in vitro* study. The findings that we can summarize are in the physical test treatments all of the groups has good quality and have not negative effect on the ruminal environment.

Keywords | Complete feed, Physical quality, Protection, Virgin coconut oil, Wafer.

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***Correspondence** | Desy Cahya Widianingrum, Department of Animal Husbandry, Faculty of Agriculture, University of Jember, Jember, 68121, Indonesia; **Email:** dsycahya312@gmail.com
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INTRODUCTION

Both of the quantity and the quality of feed are still a problem on Indonesian farms. Almost 25-50% of farmers have access to feed in the dry season, and it is lower compared to wet season (51-75%) (Widyobroto et al., 2020). In the dry season, many regions have a surplus of straw from rice, corn, and other farm by-product. The utilization of straw as feed is 40% of the total production each year (Anshar et al., 2015). Some farmers give straw as a feed directly even though the nutrition of straw is very low (crude protein: 0.05%). They also not use technology to keep it for the dry season (Syamsu et al., 2015).

Technology that is easy, inexpensive, and can increase shelf life is needed to overcome the scarcity of feed availability in the dry season (Retnani & Saenab, 2014). Wafer production from rice straw with complete nutritional content is a solution for animal feed and is one of the solutions to improve animal feed and reduce waste. The process of making wafer, in general, is carried out by compaction with pressure and heating to produce wafer of the same shape and size. The raw material used consists of fiber sources, namely forage and concentrate, with compositions arranged based on the nutritional needs of livestock (Kurniawan et al., 2019). This product also can be used as feed in the distribution of livestock process.

This study used VCO as a supplement in the ruminant's complete wafer feed. It knew that the addition 2-8% of VCO in the feed could decrease methane production (18.39% - 29.7%) (Yuniwarti et al., 2013) and 14.5% in condensed VCO with tanin (Sondakh et al., 2015). The use of VCO 2% - 8% as ruminant feed was reported to reduce methane production 18.39% - 29.7% (Yang et al., 2016) by modifying microbial population structure in the rumen (Cusiayuni et al., 2019). VCO as a feed supplement in ruminants may be dangerous for many species of rumen bacteria, especially for fiber-digesting bacteria. This is because fat can wrap the feed particles so that it closes the surface access of the cell membrane. Microbes must stick to feed in the process of degradation. The presence of fat inhibits the attachment of bacteria to feed able can also interfere with the production of enzymes to degrade feed so that it affects on the decreasing feed digestibility (Benchaar et al., 2011). Therefore, we need protection for feeding VCO for ruminants with the protection process.

We used protective virgin coconut oil (VCO) as one of the components of the wafer (P1: saponification, P2: formaldehyde protection, P4: tannin protection) compared with the control wafer with VCO without protection (P0) and wafer without VCO (P3). For maintain the essential fatty acids contained in VCO, we also did not use the heating process to make wafer. This innovation is expected to be a solution to problems in the field of animal husbandry and agriculture, as well as support the creation of a comprehensive food safety system for livestock.

MATERIALS AND METHODS

This study did not use experimental animals. The ration consisted of 30% rice straw, 30% concentrate, 30% indigofera, 5% molasses, and 5% VCO (except P3). The group of treatments were wafer supplemented with VCO (P0); wafer supplemented with VCO saponification (P1); wafer supplemented with VCO protected by formaldehyde (P2); wafer without VCO (P3); and wafer supplemented with VCO mixed on *indigofera* (P4). Each treatments were using six replications and tested for their physical quality, rancidity, and rumen fermentation characteristics and *in vitro* digestibility.

Saponification VCO procedure

Protection of fat by saponification using a solution of NaOH (Merck, Germany) and $CaCl_2$ (Merck, Germany). The balance for oil: NaOH 5%: $CaCl_2$ 0.25% is 4: 1: 1 (Wulandari et al., 2019).

The procedure of making wafer

The process of wafer production was conducted by chopping, drying, mixing, preassing, and forming with a manual hydraulic without heating. The wafer formula consist of 30% sun-dried indigofera, 30% rice straw, 30% concentrate (Indofeed SP 25), 5% molasses, and 5% VCO (except on P3).

The physical quality test of wafers

The physical properties test consists of density test, water absorption, thickness development, and texture hardness test.

Density test*:* The sample was weighed then measured in diameter and thickness (Trisyulianti et al., 2003). The calculation uses the following formula:

Density (g/cm^3) =
W = sample weight (g)
V = sample volume (cm^3)

Water absorption: Samples were weighed before and after immersion for 5 min (Trisyulianti et al., 2003). The calculation uses the following formula:

Water absorption
W1 = weight before immersion (g)
W2 = weight after immersion (g)

Thickness swelling: Samples were measured thick before and after immersion for 5 min (Trisyulianti et al., 2003). The calculation uses the following formula:

Thickness swelling
T1 = thickness before immersion (cm)
T2 = thickness after immersion (cm)

Hardness of texture: Texture hardness was measured using a Precision Penetrometer 1/10 mm division (GCA, Precision Scientific Chicago Illinois) (Boodhoo et al., 2009). Place the cone-shaped needle on the sample surface then the locking clamps were opened for 10 s. Note the

depth of the needle piercing material. The calculation uses the following formula:

G1 = final depth
G2 = initial depth

Rancidity test

The rancidity test was done by weighing a sample of 0.01 g, then inserting it into a threaded test tube, adding 1 mL of TBA reagent, then heating to a temperature of 100° C for 15 min. The solution was allowed to cool, then add 3 mL of 96% ethanol and 1 mL of isobutanol. The solution was measured at absorbance with a wavelength of 535 nm (Shimadzu, Japan). Rancidity value can be determined by calculation (Manoj et al, 2020):
Rancidity (mmol/kg) = Acm^{-1}

In vitro digestion test

In vitro digestion test based on the two-stage method (Tilley & Terry, 1963), that has been modified where without residual leaching was carried out in the stage I procedure to stage II, so that the addition of HCl (Merck, Germany) and pepsin was done immediately Merck, Germany). The wafer was ground and then as much as 500 mg fermented in 50 mL in vitro syringe (Pyrex, New York), which was filled with one part of rumen fluid and four parts of artificial saliva or buffer solution that functions as an artificial rumen and then given CO2 to create anaerobic conditions and incubated in the water bath with temperature 39° C (Ollital, China). The first stage lasts 48 h. The second stage is the abomasum condition made by adding 20% HCl to each test tube as much as 3 mL (by way of giving in stages 0.5; 0.5; 1; 1 mL), and after that five persent pepsin was added as much as 1 mL. Digestion in the abomasum lasts for 48 h. Every incubation point from the incubated treatment, blank, and standard was replicated five times. Blank is a tube that is filled without treatment samples, its function is as a correction factor. Standard is a tube filled with pangola grass. Shaking out is done manually every eight h. The process of absorption of food substances in the small intestine is mimicked by filtering samples that have undergone fermentation in the artificial rumen and abomasum. The parameters tested included dry matter digestibility (DMD), organic matter digestibility (OMD), pH, and NH_3.

Data Analysis

The experimental design used in this study was a completely randomized design (CRD) with five treatments. Six replications were used for physical quality, rancidity, and rumen fermentation characteristics. Digestibility data was tested in four units (each unit was replicated five times). The data obtained were analyzed using variance analysis (ANOVA) and further tested with honestly significant difference (HSD) (SPSS statistical software version 21.0; SPSS Inc., Chicago, IL, USA).

RESULT AND DISCUSSION

Physical quality test of wafer

The results of the physical quality test of the wafer are presented in Table 1. Based on the data obtained it was known that the density value on the P4 wafer (0.23 ± 0.16 g/cm3) does not differ significantly from the P0 (0.208 ± 0.11 g/cm3), P2 (0.207 ± 0.13 g/cm3), and P3 (0.20 ± 0.09 g/cm3) and significantly different from P1 (0.198 ± 0.15 g/cm3). The density value affects the shape and density of the wafer. The wafer density value of study's results showed that the water was not too hard, so the ruminants easily consumed it. P1 group was not significantly different from P0, P2, and P3, showing that the chemical content of the saponification does not have a major effect on the density value. Wafer P4 has the highest density. Until now, there is no standard quality of animal feed wafer. Wafer with high density may not be preferred by livestock because they are hard and difficult to consume.

Wafer with density values greater than 0.7 g/cm3 were less palatable when consumed by livestock (Retnani et al., 2014). There was no ideal standard also for the density value in feed. The density of the wafer determines the physical form of the complete wafer ratio, which can be seen from the density level (Fuadi, 2013). This value is influenced by the formulation and moisture content of the constituents and the manufacturing process. Pressure in the process of making wafer determines the level of bonding between material particles, so the higher the pressure can increase the density value (Retnani et al., 2014).

The wafer of P1 (77.60 ± 0.07%) and P4 (68.55 ± 0.02%) have the high water absorption. The water absorption in this treatment group was caused by the VCO fat content that has been bound to the protection agent. The yield on the P1 group was higher than P3 group (72.43 ± 0.08%), illustrates that particles between materials P1 were more porous than P3, seen from the higher P3 density values than P1. This also occurs in P2 and P0, where high densities cause low water absorption values. Low absorption at P0 was also possible because the VCO was mixed without going through the protection process. So that the nature of water and oil that cannot be fused makes the water absorption at P0 low. Wafer P4 has a high absorption with a high-density value. Bonding between feed particles with VCO content that has been bound to indigofera allows the formation of thick wafers that are dense but easily absorb water.

Water absorption is a variable that shows the ability of wa-

fer to draw water around it (air humidity) to bind to

Table 1: The physical quality of wafer complete feed

Treatment	Density (g/cm³)	Water absorption (%)	Thickness swelling (%)	Texture hardness (mm/10 s)
P0	0.208 ± 0.11[a]	57.30 ± 0.14[a]	12.95 ± 0.04[e]	41.20 ± 0.14[b]
P1	0.198 ± 0.15[a]	77.60 ± 0.07[b]	10.69 ± 0.10[b]	52.20 ± 0.14[d]
P2	0.207 ± 0.13[a]	66.67 ± 0.02[ab]	11.63 ± 0.05[c]	46.61 ± 0.11[c]
P3	0.200 ± 0.09[a]	72.43 ± 0.08[ab]	10.32 ± 0.01[a]	52.19 ± 0.14[d]
P4	0.230 ± 0.16[b]	68.55 ± 0.02[ab]	12.28 ± 0.05[d]	31.68 ± 0.22[a]

Note: Differences in notation show significantly different at 5% confidence level ($P < 0.05$)
P0: wafer supplemented with VCO, P1: wafer supplemented with VCO saponification, P2: wafer supplemented with VCO protected by formaldehyde, P3: wafer without VCO, P4: Wafer supplemented with VCO mixed on indigofera

Table 2: The rancidity test of wafer complete feed

Treatment	Rancidity (mmol/kg)
P0	2.39 ± 0.01[c]
P1	2.45 ± 0.02[c]
P2	2.58 ± 0.02[d]
P3	2.04 ± 0.01[a]
P4	2.14 ± 0.09[b]

Note: Differences in notation show significantly different at 5% confidence level ($P < 0.05$)
P0: wafer supplemented with VCO, P1: wafer supplemented with VCO saponification, P2: wafer supplemented with VCO protected by formaldehyde, P3: wafer without VCO, P4: Wafer supplemented with VCO mixed on indigofera

Table 3: Nutritional content of wafer complete feed

Treatment	Water content	Crude Protein	Crude Fat	Crude Fiber	Ash	Ca	P	*TDN
	%							
P0	13.59	18.15	6.94	13.04	11.05	0.56	0.23	73.41
P1	13.53	18.21	7.19	14.86	11.25	0.47	0.22	71.49
P2	13.88	18.54	6.61	13.25	11.42	0.45	0.22	72.29
P3	13.94	18.57	4.02	13.55	11.55	0.09	0.19	69.23
P4	13.74	18.44	6.71	14.22	11.38	1.40	0.22	72.81

Note: *Calculated by the formula Total dygestible nutrient (TDN) = 2.79 + 1.17 crude protein + 1.74 crude fat - 0.295 crude fiber + 0.810 extract material without nitrogen (Sutardi, 2001).
P0: wafer supplemented with VCO, P1: wafer supplemented with VCO saponification, P2: wafer supplemented with VCO protected by formaldehyde, P3: wafer without VCO, P4: Wafer supplemented with VCO mixed on indigofera

Table 4: Results of *in vitro* digestibility and characteristic of rumen conditions

Treatment	P0	P1	P2	P3	P4
DMD stage I (%)	64.84 ± 3.76[b]	51.62 ± 2.84[a]	60.88 ± 1.89[b]	61.63 ± 0.61[b]	72.38 ± 0.28[c]
DMD stage II (%)	78.89 ± 0.06[b]	78.24 ± 0.09[ab]	76.83 ± 1.55[a]	77.76 ± 0.58[ab]	76.77 ± 0.53[a]
OMD stage I (%)	62.14 ± 3.38[cd]	48.66 ± 1.33[a]	55.51 ± 3.41[b]	59.19 ± 3.75[bc]	68.02 ± 2.06[cd]
OMD stage II (%)	78.49 ± 0.16[b]	77.80 ± 0.04[ab]	75.46 ± 2.34[a]	77.30 ± 0.02[ab]	77.00 ± 1.23[ab]
pH	6.85 ± 0.105[a]	7.13 ± 0.15[b]	7.15 ± 0.12[b]	7.11 ± 0.15[b]	6.96 ± 0.12[ab]
NH_3 (mg/100 mL)	18.34 ± 0.19[ns]	18.76 ± 0.67[ns]	17.82 ± 0.47[ns]	17.71 ± 0.45[ns]	17.64 ± 0.17[ns]

Note: Differences in notation show significantly different at 5% confidence level ($P < 0.05$)
DMD: dry matter digestibility
OMD: organic matter digestibility
P0: wafer supplemented with VCO, P1: wafer supplemented with VCO saponification, P2: wafer supplemented with VCO protected by formaldehyde, P3: wafer without VCO, P4: Wafer supplemented with VCO mixed on indigofera

material particles or be held in the pores between material particles. Wafer with too high a moisture content can fa-

cilitate bacterial growth. However, high water absorption helps wafer to be soft when exposed to livestock saliva when chewed (Retnani et al., 2010).

The wafer thickness value from the lowest to the highest consecutively was P3 (10.32 ± 0.01%), P1 (10.69 ± 0.10%), P2 (11.63 ± 0.05%), P4 (12.28 ± 0.05%) and P0 (12.95 ± 0.04%). Wafer from P0, P2, and P4 groups was softer with high thickness development indicators in line with lower hardness test results. Based on the results, the hardness of the P3 (52.19 ± 0.14%) group was not significantly different from P1 (52.20 ± 0.14%). Wafer P1 has high water absorption and hardness, so it is not easy to crumble and can maintain its shape according to the mold when packing and removing the wafer.

Thickness swelling is the ability of wafer to expand when absorbing water. High thickness swelling indicates that softening of the wafer when consumed by livestock. Wafer that has a high thickness development will make the wafer softer, making it easier for cattle to chew, so it will increase livestock consumption (Retnani et al., 2014). Hardness test on wafer is related to the ability of livestock teeth to chew wafer. The lower the water content, the higher the texture hardness and crispness will increase (Retnani et al., 2010).

The process of making wafer in this study uses a manual hydraulic press machine without heating. The purpose of compression without heating was to maintain the content of essential fatty acids in VCO. Making wafer has been done optimally both from the preparation of the material and the pressing process (Retnani et al., 2014).

Rancidity test

The results of the rancidity test were presented in Table 2, and it showed that the wafer P3 (wafer without VC) has the lowest rancidity (2.04 ± 0.01 mmol/kg). The P4 wafer supplemented with VCO mixed on Indigofera (2.14 ± 0.09 mmol/kg) was significantly higher than P0 (2.39 ± 0.01) and P1 (2.45 ± 0.02). Wafer supplemented with VCO protection using formaldehyde was found to have the highest rancidity values. These data indicate that the fat oxidation in wafers supplemented with VCO protected by Indigofera runs slower than VCO protected by saponification and formaldehyde. Indigofera reported having higher Lipoxygenase inhibiting activity than can inhibit the lipoxygenase enzyme action (Rahman et al., 2018). The lipoxygenase inhibitor has a role in inhibiting the Lipoxygenase catalyzes fatty acid hydroperoxides and is associated with rancidity, aroma properties production, color changes, and alternation of physicochemical attributes (Shi et al., 2020). The saponification treatment also lowers the rancidity of the wafer compared to unprotected VCO. The treatment of saponification also has lower rancidity than unprotected. The saponification might be modified the pH value. The modulation of pH using calcium salt can lower lipase activity and peroxidase value (Mohammadi et al., 2021). However, the rancidity is influenced by several factors such as fatty acid composition (double bond, faster oxidation), oxygen concentration, temperature, surface area, transition metal, and enzyme activities (Poiana et al., 2021). Moreover, the packaging material and process also affected in peroxidation process during storage and distribution (Cestari et al., 2015).

The nutritional content of wafer

Nutritional content of wafer is presented in Table 3. The rations on complete wafer feed were iso crude protein (18.15% to 18.57%) with a total digestible nutrient (TDN) content ranging from 69.23% to 73.41%. The complete wafer feed in this study can be used to meet the needs of ruminant animals for dairy cattle and beef cattle (Indonesia National Standards, 2009). In general, ruminants need protein content of 12% to 14% and TDN 55% to 65% depending on the type of production and physiological status.

In vitro digestibility test and characteristics of rumen fermentation

The results of *in vitro* digestion and an overview of rumen conditions were presented in Table 4. *In vitro* digestion stage I represented the digestion process in the rumen, whereas stage II represented total digestibility. The results of dry matter digestion stage I showed that P4 (VCO mixed with indigofera) had the highest digestibility, and P1 (saponified VCO) had the lowest significant digestibility. Meanwhile, P0 (VCO), P2 (protected with formaldehyde), and P3 (wafer without VCO) were not significantly different. The high P1 digestibility value is possible because the VCO binds to the tannins contained in indigofera. Tannins did not bind with feed protein but VCO, which does not reduce the total dry matter digestibility. This bound makes VCO not degraded in the rumen and will be digested in the small intestine.

The low digestibility of the VCO treatment saponified showed that there was a fermentation disorder which ultimately caused the microbes not to work optimally. However, the total dry matter digestibility (stage II), showed that there was no significant difference in the P0, P1, and P3 treatments. Interestingly, in P4 which is the highest stage I, in stage II has the lowest significant value among other treatments. The presence of tannins in P4 may have a positive effect on binding VCO and a negative effect on binding to feed protein after the VCO-tannin bond is released in the abomasum due to acidic conditions. This causes a decrease in the digestibility of dry matter P4.

The digestibility value of P2 (protection with formalde-

hyde) stage II was significantly lower than P0 but at stage I was higher than P1 indicating that formaldehyde treatment caused a more conducive rumen atmosphere than the saponification treatment. In this research, formaldehyde treatment was used only as comparison or control data because it has succeed protected VCO (Widianingrum et al., 2019). In their study, VCO protected with 2% formaldehyde showed the best results seen from the DMD and OMD values, rumen fluid pH, proportion (Ascetic + Butyric): Propionic, NH_3 concentration, and microbial protein concentration.

The use of formalin, although it is successful in protecting VCO, is not recommended and may be dangerous, so alternative methods and other materials are studied. Another method, for example is saponification. Saponification is used for rumen bypass technique in feeding so that the protected substance is not degraded by rumen microbes and can be digested in the abomasum and small intestine without interfering with the fermentation process in the rumen (Pramono et al., 2018). This process occurs due to emulsification between feed molecules and soap molecules. Soap is easily suspended in water because it forms micelles, a group of (50 to 150) molecules whose hydrocarbon chains cluster with the ends of ions facing the water (Saha et al., 2018). The process of protection with indigofera can occur because of the tannin or saponin content in the feed. Tanin-bound or saponification fats are not degraded by rumen microbes so that they pass from the rumen to the abomasum. The bond will break when in the abomasum due to an acidic environment. The method of mixing VCO with indigofera allows bonds between particles of material to be more vital to produce the highest density value. Feed protection is carried out to protect certain nutrients with the principle of bypass rumen (Rodríguez et al., 2016).

There was no harmful effect in the rumen with the addition of VCO without protection (P0) at *in vitro* study. Seen from the DMD and DMO values of level I, which were higher than the treatment without VCO (P3). This finding contradicts the results of the *in vivo* study described in Widianingrum et al. (2019) that VCO without protection affects the condition of the rumen, which drastically reduces the body weight of livestock (Noviandi, unpublish data).

Low ammonia concentrations in rumen fluid can reflect that the fermentation process is going well so that ammonia is appropriately utilized, protein diets are difficult to degrade, or protein content is low. High stage I digestibility values in feed can illustrate that the presence of VCO does not have a negative influence on rumen microbial activity. This can be seen from the rumen pH and NH_3 value in this study. Provision of VCO reported that it does not affect rumen microbial activity (Widianingrum et al., 2019). The pH value in the rumen can be maintained in the range of 6 to 7 because of the presence of salivary secretions that function as a buffer. This is one indicator of the process of good feed degradation. The appropriate pH conditions support the process of rumen microbial metabolism so that the digestion process of feed ingredients can run optimally (Aschenbach et al., 2011). Ammonia is the main source of nitrogen for microbes to synthesize amino acids for their development. Measurement of ammonia production is used to illustrate the ability of microbes to use it (Pramono et al., 2018).

CONCLUSION

The findings that we can summarize in this study were in the physical test, and all treatments had standard wafer feed quality. The advantage of VCO protection treatment on wafers is in the lower rancidity value compared to wafers with VCO supplementation without protection. Our results showed that there was no effect on digestibility and the rumen environment, such as pH and NH3 levels produced both protective and non-protective VCO.

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CONFLICT OF INTEREST

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service, and the company that could be construed as influencing the content of this paper.

NOVELTY STATEMENT

This research used a non-heating method for making a wafer for feed. This method can maintain the essential component of virgin coconut oil. This product can use as feed during distribution and also for daily consumption.

AUTHORS CONTRIBUTION

Desy cahya Widianingrum: concepts, design, experimental studies. Himmatul Khasanah: Literature search, manuscript editing. Melinda Erdya Krismaputri: experimental studies, data analysis. Susan Barbara Patricia Sembiring

Meliala: manuscript preparation, manuscript review.

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