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Review Article





Challenges in the Definition and Measurement of Subacute Ruminal Acidosis in Holstein Dairy Cows: A Review

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ABSTRACT

Subacute ruminal acidosis can be defined as a depression of rumen pH, which affects animal health and production. Although researchers have tried to find a solution for this disorder, it is a prevalent problem that causes considerable losses in commercial dairy cow production. This review aims to reveal critical points in current knowledge about subacute ruminal acidosis and suggest solutions for future research. The first challenging issue in subacute ruminal acidosis is the diagnosis method of this disorder, which requires appropriate statistical evaluation and modeling. In addition, biological factors should be considered to define subacute ruminal acidosis since some roles have recently been observed for different CO₂ species in the rumen as a direct cause of the events. These CO2 species are sometimes more accurate than rumen pH in explaining the decrease in feed intake, milk yield, milk fat percentage, and inflammation responses. In the future, the measuring of the CO_2 species in the rumen may be a replacement for pH measurement or become a factor that can greatly explain ruminal acidosis. Compared to basic methods, another challenging point is the reliability of rumen pH measurements as well as the accuracy of newly developed sensors. The reticular pH with current boluses could be measured by monitoring cows on-farm or a large number of animals in research. In conclusion, a thorough definition and precise application of new measurement devices can reveal some unknown factors for subacute ruminal acidosis in dairy cows.

1. Introduction

A reversible depression of rumen pH, which influences animal production and health can be defined as subacute ruminal acidosis^{1,2}. Subacute ruminal acidosis has been known as a predominant disorder in ruminants and it has been experimented to find a solution for around a century³. Although the efforts resulted in several recommendations as review or extension works, this syndrome is still one of the major problems in dairy farms⁴. The known symptoms of subacute ruminal acidosis, such as disruption of health, production, and well-being of animals are still prevalent^{5,6,7} resulting in enormous economic losses^{8,9}. Rojo-Gimeno et al. in a quantitative study reported an average true prevalence of 16% of the herd, which resulted in 210 euros/case/year losses as the cost of disease and 200 - 250 euros/case/year as the cost of treatment⁸. A recent survey on a total of 32 commercial dairy farms in Australia, the United States, and Canada reported that on average 26% of animals were at high risk of ruminal acidosis⁴. Failure of prevention of such high losses may have been due to some unknown or missing factors in the science of subacute ruminal acidosis and the current knowledge needs to be reevaluated and re-analyzed carefully^{2,10,11}. This concept became the objective of some recent research that reevaluated subacute ruminal acidosis for challenging points, such as definition and nomination¹²⁻¹⁵ the role of different CO₂ species as real causes^{10,16}, and method of measurement^{17,18,19}. However, to open new perspectives on this topic, it was necessary to summarize, compare, and evaluate the results of previous studies in a review article. Therefore, the objective of the present

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review was to summarize and evaluate the outcomes of recent studies that evaluated the causes, definition, and measurement of ruminal acidosis, and propose some perspectives for the future.

2. Ruminal acidosis, a blind story

2.1. The complex nature of the disease

More than 70 years ago, some dietary failures related to the condition of ruminal acidosis were discovered, which caused several disturbances in terms of health and performance³. To explain the condition of dairy cows, it was called acute (acid) indigestion by previous researchers^{3,20} and later due to the complex nature of this problem in both causes and effects and for simplifying, it as subacute ruminal acidosis³. Then was known researchers tried to make a definition for this disease. At the experimental level it was defined as a time or area of pH below a certain point and at the commercial dairy farm level it was defined by a value of rumen pH sampled through the Rumenocentesis method (Table 1). However, as reported in Table 1 each research infrastructure (center) defined subacute ruminal acidosis based on what was inferred and a lot of disagreements were reported to define the problem (The references are cited in Table 1).

The complex and variable nature of the so-called subacute ruminal acidosis in both causes and side effects are important constraints to making a consistent definition. In one vision, various definitions of the socalled subacute ruminal acidosis could be related to the way it was caused. Failure in diet adaptation¹², feed restriction¹³, forage particle size¹⁴, and the concentrate level in the diet¹⁵, are known as practical ways and each in turn can be described in several ways. In another vision, the variation in the definition of the so-called subacute ruminal acidosis could be related to the value of pH and the considered side effects, including milk fat depression^{15,18}, the depression of fiber digestion in the ruminal²¹, as well as inflammation²². Nomination of the so-called subacute ruminal acidosis may not discriminate and define the real problem in both causes and effects. Sometimes, due to the misunderstanding of the farms or cow's problem, it is simply claimed as acidosis by managers, scientists, or professionals. So, in practice, it is defined as a blind nomination.

2.2. Lipopolysaccharides release and relative inflammation

It is demonstrated that depending on the causes (forage particle size or concentrate level), low rumen pH can have different consequences to affect animal health and performance. Li et al. conducted an experiment on inducing low rumen pH by feeding pellets of alfalfa or pellets of barley and wheat grains²³. Although, both inducing methods dropped rumen pH and raised time and area of рΗ under 5.8 in a relatively similar way. lipopolysaccharides (LPS) concentration in digestive fluid (rumen, cecum, and feces) was 5 times more when low rumen pH was induced by grain pellets versus alfalfa pellets. Accordingly, a conduction of two experiments from the same team indicated that with a similar severe induction of low rumen pH, grain-based induction²² as opposed to induction by alfalfa hay pellets²⁴ may result in a visible amount of LPS in plasma.

The two ways of inducing low rumen pH might be related to different ways of acid production and accumulation in the rumen. Indeed, feeding alfalfa pellets may decrease chewing and reduce the supply of buffers from saliva²⁵ while grain pellets increase acid by sudden change in rumen fermentation²². Therefore, inducing low rumen pH with alfalfa pellets apparently had less effect on rumen microbes than grain-based induction and, consequently it was not accompanied by a change in Escherichia coli bacteria population and its specific toxins, such as LPS²⁶. Indeed, ingestion of the extremely high-fermentable diet by feeding high-concentrate and low-fiber diets changed the rumen microbial ecosystem²⁷, consequently toxin (LPS) was released, and toxicity (inflammation) happened^{1,2}.

The grain-based induction of low rumen pH by feeding a high amount of starch can also cause starch transfer to the hindgut, but inducing by feeding alfalfa pellet did not². The hindgut fermentation of starch and relative dropping of pH also might be responsible for augmenting LPS concentration in those organs, as well as in the plasma in grain acidosis induction compared with alfalfa acidosis induction²³. It is also documented that grain-based induction of low rumen pH increased *E. coli* in the hindgut but not in the rumen²⁸. Recently, it was reviewed that the inflammatory response of LPS originating from rumen bacteria may be less effective than LPS originating from *E. coli*^{29,30}. Calsamiglia et al.

Table 1. Some examples of variation in the definition of subacute ruminal acidosis in Holstein dairy cows between and within different methods of measurement

Study	Method of measurement	Definition
Garrett et al. (1999); Krause and Oetzel (2006)	Rumenocentesis	A rumen pH ≤ 5.5 measured approximately 4-10 hours after feeding
Nasrollahi et al. (2017)	Rumenocentesis	A rumen pH < 5.8 measured at 4 hours after feeding
Vallejo-Timarán et al. (2020)	Rumenocentesis	A rumen pH < 5.6 (measuring time not mentioned)
Nasrollahi et al. (2017)	Continuous reticuloruminal pH	A duration of reticuloruminal pH < 5.8 for > 330 min per day
Jing et al. (2018)	Continuous reticuloruminal pH	A duration of reticuloruminal pH < 6.0 for > 360 min per day
C_{00} at al. (2010)	Continuous reticuloruminal pH	An acidosis index > 0.5 for rumen pH = 5.8; A value comparable with
60011 et al. (2019)		A duration of reticuloruminal pH \leq 5.8 for \geq 180 min per day
Yang et al. (2022)	Continuous reticuloruminal pH	A duration of reticuloruminal pH < 6 for \geq 180 min per day
Zhao et al. (2018)	Continuous rumen pH	A duration of pH between 5.2 and 5.6 for ≥ 180 min per day
AlZahal et al. (2007); AlZahal et al. (2014)	Continuous rumen pH	A duration of rumen pH < 5.6 for ≥ 300 min per day
Zebeli et al. (2008)	Continuous rumen pH	A duration of rumen pH < 5.8 for > 314 min per day

Study	Khafipour et al. (2009a) ^{1,2}	Khafipour et al. (2009b) ²	Zhao et al. (2018)²	Steele et al. (2012) ⁴	Krause and Oetzel (2005) ^{3, 2}	Pourazad et al. (2016) ^{3,5}	
Inducing model (value are DM based)	Replacing chopped alfalfa with alfalfa pellets gradually and during 5 weeks	Replacing 21% TMR with grain pellets, abruptly and during 1 week	switching from 50 to 60% concentrate, abruptly and during 5 weeks	switching from 11 to 62% concentrate, abruptly and during 3 weeks	Adding 3.5 - 4.6 kg of grain pellets to the diet abruptly and during 1 day following 1 day feed restriction	switching from 0 to 60% concentrate, gradually and during 4 weeks with one week break	
mean	5.78 vs. 6.35**	5.97 vs. 6.17*	5.95 vs. 6.51 ^a	5.82 vs. 6.18**	5.85 vs. 6.31**	5.93 vs. 6.38**	
Duration of pH < 5.8, [min/d]	447 vs 112**	279 vs. 118*	369 vs. 0ª	712 vs. 93**	496 vs. 66**	497 vs. 32 **	
Area PH < 5.8, [min × pH/d]	69 vs. 24	102 vs. 15*			190 vs. 15**		
DMI, [kg/d]	23.4 vs. 18.1**	16.5 vs. 19**		18.1 vs. 19.4**	27.9 vs. 25.2**	15.2 vs. 11.0**	
MF[%]	2.32 vs 3.22**	2.93 vs. 3.30**		3.25 vs. 3.89*	4.29 vs. 3.73*		
Acute phase protein in blood (plasma or serum), μg/mL							
LPSBP	3 vs. 7	53 vs. 18*	40 vs. 20**				
SAA	7 vs 23*	447 vs. 164*	370 vs. 110**				
HP	12 vs 56**	484 vs. 0**	300 vs. 180**				
Rumen VFA (mM)							
Acetate	67 vs. 54**	54 vs. 62*	88 vs. 66**	49 vs. 48		59 vs. 66**	
Propionate	40 vs. 22*	35 vs. 22**	24 vs. 16*	27 vs. 20**		24 vs. 19**	
Butyrate	12 vs. 11	15 vs. 11*	17 vs. 11**	15 vs. 12**		12 vs. 9.5**	
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Table 2. Some of the dietary models for inducing subacute rumen acidosis and relative responses of dairy cows (All values are mean values of induced versus control)

DM: Dry matter, DMI: Dry matter intake, MF: Milk fat, LPSBP: Lipopolysaccharide binding protein, SAA: Serum amyloid A, HP: Haptoglobin, VFA: Volatile fatty acids, vs: Versus

¹ Data on the final week of inducing were reported here

² In these experiments pH 5.6 was used instead of 5.8

³ In these experiments VFA was the molar percentage

⁴ Data from the final week of inducing were reported here

⁵ Data of the final episode of the transient model were reported here

* indicates on P values ≤ 0.05 and ** indicates on P values ≤ 0.01

^a No statistical comparison.

indicated the events that happen during feeding high concentrate (grain) diets cannot be fully explained by the drop of rumen pH and they suggested using the name of high-concentrate syndrome instead of sub-acute rumen acidosis³¹.

It is worth mentioning that accurate measurement of LPS with different techniques may affect the outcome and future experiments need to be taken into account. Indeed, it is suggested to provide a standard method to increase the accuracy and precision of the results in different experiments.

2.3. Variation in protocols of experimentally inducing the disease

Another area of problem for the definition of the socalled subacute ruminal acidosis is related to the way it was induced experimentally. Adding various amounts of pellets of highly fermentable grains²², or alfalfa²⁴ to the diet, restricted feeding followed with grain challenge³², or sudden raising of concentrate level³³ were regular ways of inducing the disease, and depending on these ways the consequences were varied. Table 2 summarizes some of these protocols and indicates their variable responses on rumen parameters, dry matter intake, milk fat percentage, and inflammatory response in the blood (acute phase proteins). Limited biological explanations or quantitative analyses have been conducted for these approaches to show certain side effects of low rumen pH. Another problem of these approaches is the inducing length and sampling time which occasionally was limited to 1-7 days^{22,23,32}. It has nothing to do with the regular condition of long-term feeding of high concentrate diets^{9,34} or highly digestible plant species in pasture³⁵ in practice. Stauder et al. tested switching dairy cows' diet from a high forage [60 % of dry matter (DM)] to low forage (40 % of DM) and monitored cows for 2 weeks before and 4 weeks after the switch¹⁵. Evidentially, almost all the changes in rumen pH and inflammatory biomarkers were limited to the first week after switching, and in the next weeks, both low and highforage diets showed similar values with different milk fat percentages. In order to make a sensible definition of inducing ruminal acidosis, the expected amount and time of dropping of pH and objective response (depression of fiber digestion, milk fat reduction, or inflammation) need to be indicated.

2.4. Perspectives

It is evident that the definition of the concept of subacute ruminal acidosis is not comprehensive, conclusive, or exclusive and the definition needs to be revised to be more accurate and informative for researchers and practical users. Regarding the Complex nature of the disease, providing an appropriate definition is a complex task and needs a deep mental and statistical analysis and modeling. A suggestive approach to give such a definition would be achieved by a meta-analysis of the literature and by that low rumen pH could be

categorized based on the intensity, causes, as well consequences, and accordingly a standard definition could be developed. Depending on the nature of the consequences, the corresponding pH could be a point (for threshold treats) or a range (for quantitative treats). Based on that definition, inducing low rumen pH also makes more sense by using a specific way of inducing as cause and expected specific consequence- low rumen pH by minimum amount of grain pellets for minimum significant augmenting of inflammation in plasma. In this manner, when researchers try to induce low rumen pH and seek a specific response a standard approach would be available. These quantitative perspectives can help practical users understand and treat subacute ruminal acidosis problems more precisely. Moreover, to provide such a commonplace definition an agreement among several research centers and scientific societies is needed. In this manner, the final stranded will be followed worldwide. Up until the time that this model is developing, it is preferable to avoid the blind term of subacute ruminal acidosis and it is better to be more specific with terms like low rumen pH diseases (if rumen pH is measured) or high concentrate diets diseases (if just this diet fed without measuring rumen pH).

3. Challenges with measurement of the rumen pH: Accuracy and precision of different methods

3.1. History

Several research and review studies have been published about the accuracy and precision of different methods to measure rumen pH^{1,36,37,38,39}. Overall, continuous measurement of rumen pH is advantageous over spot sampling^{1,39}, and measuring by rumenocentesis is advantageous over stomach tubing since it is not contaminated by saliva^{36,37}. There were some health issues with the rumenocentesis technique at the beginning^{40,41} but recent findings indicated no serious health problems by this method^{42,43,44}.

3.2. Ups and downs of rumen sensors and telemetry (wireless) transmission of data

Recently, a new generation of pH measuring methods has evolved to measure the continuous reticular pH of nonfistulated animals⁴⁵. The new generation of rumen pH monitoring devices have the capacity of long-term-auto calibrated monitoring of rumen pH and wireless transmission of data⁴⁵. These aspects allow the potential of orally administrated devices in non-fistulated animals to provide long-term continuous measurement of reticular pH^{17,19,45}. Due to no need for fistulated animals and longterm measuring of pH, the devices became popular in the world¹⁷ but limited studies have evaluated the calibration adequacy after several days, and limited publications dealt with the accuracy of reticular pH for detecting the real condition of rumen pH³⁸. Sato conducted a comparison between the measurement of pH in the reticulum and rumen with the same type of pH meter and due to a correlation, coefficient of 0.75, it was suggested the reticulum pH can have an estimate of rumen pH⁴⁶. However, the dropping of pH due to dietary induction of low rumen pH was two times greater in the rumen than reticulum. The precision of the measurement is being discussed as both sites were measured with the same-newly-designed deceive (no standard device as control) for a long time and without checking the calibration at the end⁴⁶.

Falk et al. conducted research to compare an orallyadministrated-wireless-ebolus pH meter (eBolus, eCow Ltd., Exeter, Devon, UK) that was expected to enter into the reticulum and a regular data logger (LRCpH; Dascor Inc., Escondido, CA) that was inserted into the rumen ventral sac of the same animal through a rumen fistula¹⁹. The reticular pH on average was 0.24 units more than in the rumen and had less fluctuation. In addition, according to different days relative to calving, the difference between reticular pH versus rumen pH was in the range of -0.23 to +0.44 units indicating that depending on days in milk there is no linear relationship between rumen pH and reticular pH. It might be related to diet composition, feed intake, or both19, and the external temperatures at which the calibration and subsequent measurement are taken⁴⁷. Moreover, the deviation in the calibration of the device inserted in the reticulum might also be the case since the data of the reticulum were collected during 10 weeks with no checking of calibration during or after measurement^{19,47}. The role of diet on different pH values of the reticulum and rumen was detected in another experiment which showed high forage (100%) versus low forage (35%) diets caused on average a (0.15) versus (0.70) unit difference of pH between the reticulum and rumen. Despite a high correlation in pH values of the two sites, a particular adjustment for high-concentrate diets was recommended¹⁷. Calibration is a big concern since when the device is administered for non-fistulated dairy cows there is no possibility to check the calibration during or after the measurement to conclude a review of sensor validity³⁸. In another experiment, continuous measuring of reticular pH with wireless boluses and the pH measured by rumenocentesis indicated similar categories of low rumen pH among cows fed a high concentrate diet. The correlation coefficient of mean rumen pH between the two methods was not significant $(r = 0.37, P = 0.19)^{18}$. In conclusion, measurement of reticular pH with current boluses as a way for monitoring of the cows on-farm or a high number of animals in research cannot be replaced with direct measurement of the rumen by a pH meter calibrated.

The recommended method for measuring rumen pH is the direct measurement through the rumen fistula. Calibration and measuring pH are possible in a precise region of rumen, and the data shows the condition of rumen pH in both overall and diurnal manners accurately. However, the difficulty of handling the method, problems obtaining permission for the using an appropriate number of fistulated animals by the local animal welfare authorities, and finding enough funding to support such a costly method are important constraints. Consequently, it is suggested to perform rumen-fistulation only for experiments with essential needs. These approaches may partly diminish the constraints of the rumen pH measurement through fistula but still, the problem is the case and needs to evaluate more ideas to be suggested for future research/review contributions. Conditional that constraint is not solvable the next accurate method (indwelling boluses) is recommended.

3.3. The roles of dissolved CO2 and CO2 holdup

3.3.1. Failure in conventional parameters

The concept of subacute ruminal acidosis is further complicated when the proposed roles of rumen volatile fatty acids (VFA) and pH become deficient and inconsistent^{16,48}. For instance, VFA production is the accepted way in production of acids in the rumen^{48,49} but it was observed the rumen concentration of VFA could not explain the whole variation of acid concentration and pH of the rumen fluid^{16,48}. There is a possibility that VFA as a time-point measurement is influenced by production and absorption rate and may not be able to provide an accurate indication of animal performance and health status. However, even with continuous measurement of rumen pH, sometimes explaining the decrease in feed intake, milk yield¹⁰, milk fat percentage¹⁵, and inflammation responses²³ fail.

3.3.2. Augmenting dissolved CO2 and CO2 hold-up

Alternatively, some potential roles of CO₂ species as causing the events around subacute rumen acidosis are developed and suggested by a review paper¹⁶, then validated by an experiment¹⁰. In brief, water molecules in the liquid condition are in the form of H₃O⁺ and OH⁻ and just in the gas form, they are in the form of H_2O^{50} . Increasing pH in water solution means increasing activity of H₃O⁺ (concentration of H₃O⁺) ions and not free $H^{+50,51}$. For the same reason, the molecules of CO₂ are in the gas form but also partially soluble in water and when molecules are solubilized, they change to ionic forms named dissolved CO₂ (dCO₂; with a weak linkage between CO_2 and H_3O^+) with an acidic form of $CO_2^{10,16,52}$. Normal feeding conditions contain a low amount of dCO₂ in the rumen due to the equilibrium of rumen fluid and gas and the removal of gases by eructation^{53,54}. Indeed, in this condition, the solubility (entrance into the solution) and volatility (exit from the solution) are equal, and little amount of dCO₂ remains in the rumen fluid¹⁶. However, in the condition of feeding a high amount of fermentable organic matter or forage with very fine particles the viscosity⁵⁵ and surface tension⁵⁶ of rumen fluid may increase, and the amount of dCO₂ increases¹⁰. As long as the viscosity or surface tension is high, the dCO₂ remains in the rumen fluid and causes CO_2 hold-up. In addition, in this condition volatility of $CO_2\ is\ impaired^{10,16}.$

3.3.3. Pathogenesis of CO2 hold-up

It is documented that dCO₂ has a high biological activity and its holding up for enough time causes several pathological and nutritional problems¹⁶. At the level of rumen microbes, it is evident that the requirement of CO₂ for propionate-producing bacteria is high and they grow more at high concentrations of $dCO_2^{57,58}$. Thus the high dCO₂ condition of rumen fluid leads to a low acetate to propionate ratio^{59,60} and thereby milk fat depression⁶¹. Moreover, at a lower pH, which parallels a high amount of dCO₂, lactate-producing bacteria (*Streptococcus bovis*) becomes dominant by dehydrogenation of pyruvate to lactate⁶². Possibly the high activity of these microbes is linked to the high concentration of dCO₂¹⁶. Moreover, the longtime holding up of CO₂ may increase its gradient from rumen fluid to blood up to 10 times⁶³. It may cause passive diffusion of dCO_2 to rumen epithelial cells and blood circulation^{64,65} and thereby cause saturation of the buffering system of rumen epithelial cells and metabolic acidosis^{43,66}. There is some evidence indicating the possible role of high dCO₂ on increasing histidine and LPS in the rumen^{67,68} and destruction of rumen epithelium integrity⁶⁹. It can result in triggering inflammatory responses in pulmonary endothelial cells70,71 and smooth muscle cells^{72,73}. In addition, despite a link between low rumen pH and triggering LPS-mediated inflammation the results were not consistent^{23,74} and may indicate on possible roles of dCO₂ on inflammatory responses¹⁶.

Regarding the mentioned roles of dCO₂ and CO₂ hold-up in explaining rumen acidosis, it seems important to have a deep evaluation of the complementary/priority effect of these parameters relative to the rumen pH. To validate data on CO_2 parameters, the measurement method is critical since the partitioning of CO₂ species may not be the same in the innate rumen. Rumen of cannulated animals and samples taken manually from the rumen even from the same animal fed the same diet^{10,75}. The major cause of such inconsistency is the change in partial pressure of CO_2 in different environments⁵³ as well as handling and material added during sampling^{10,76}. Furthermore, knowledge about the time duration in which dCO₂ is high and CO₂ holds up might answer some of the blind points. Moreover, the role of dCO₂ and CO₂ hold up on the change in performance of cellulolytic bacteria and biohydrogenation of unsaturated fatty acids as other features of subacute rumen acidosis^{18,77,78} need to be evaluated. Therefore, expanding the data with further research and meta-analysis study is needed to prove and validate the role of dCO2 and CO2 in explaining subacute rumen acidosis and introducing them as a standard or complementary measure for rumen acidosis.

4. Conclusion

Despite decades of research, extensions, and practical efforts, sub-acute ruminal acidosis is still prevalent. Due to

challenges in the definition and measurement method of subacute rumen acidosis, there is a need to address the causes and effects of this disorder. To achieve a commonplace definition, appropriate statistical evaluation and modeling are required. Regarding the preciseness and accuracy of new devices, the reliability of reticular pH measurements by newly developed sensors is also challenging. The measurement of reticular pH with current boluses may be a way of monitoring cows on-farm or a large number of animals in research but it cannot be replaced with direct measurement of fistulated rumen by a pH meter calibrated daily. A thorough definition and precise application of new measurement devices can reveal some unknown factors for subacute ruminal acidosis in dairy cows.

Declarations *Competing interests*

The authors have declared that no competing interests exist.

Authors' contributions

Conceptualization, writing, and editing were carried out by Sayyed Mahmoud Nasrollahi. The last version of the manuscript was read and approved by the author.

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Availability of data and materials

Data from the present study are available by reasonable request from the corresponding author.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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