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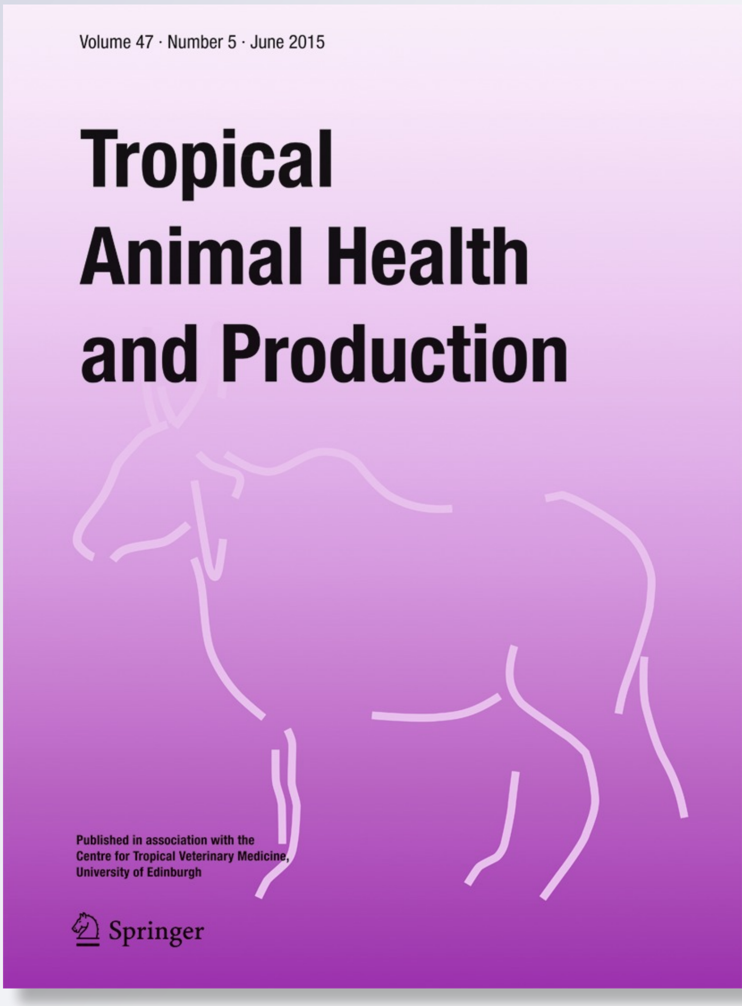

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REGULAR ARTICLES

Diet inclusion of devil fish (*Plecostomus* spp.) silage and its impacts on ruminal fermentation and growth performance of growing lambs in hot regions of Mexico

Eduardo Tejeda-Arroyo[1] · **Moisés Cipriano-Salazar**[1] · **Luis Miguel Camacho-Díaz**[1] · **Abdelfattah Zeidan Mohamed Salem**[2] · **Ahmed Eid Kholif**[3] · **Mona Mohamed Mohamed Yasseen Elghandour**[2] · **Nicolas DiLorenzo**[4] · **Blas Cruz-Lagunas**[1]

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Abstract The aim of this study was to evaluate the inclusion of devil fish (*Plecostomus* spp.—DF) silage in Criollo× Blackbelly lamb diets in hot region of Guerrero state of Mexico. Rumen fermentation including pH, volatile fatty acids (VFA) and ammonia-N (NH_3-N) and productive variables including feed intake (FI), average daily gain (ADG), and feed conversion were determined. Twenty lambs with 18±1.2 kg body weight in a completely randomized design were fed a total mixed ration (TMR) of concentrate (based on soybean meal, whole oat hay, ground corn cob, vitamins-minerals supplement) with DF silage at 0 % (DF0), 9 % (DF9), 18 % (DF18), and 27 % (DF27) of the TMR for 75 days. The ruminal pH showed no difference ($P>0.05$) between treatments: ranging between 6.21 and 6.36. Propionic acid molar proportions showed an irregular pattern between experimental groups, which only differed ($P<0.05$) between DF9 and DF27, without differences between the other treatments. A greater molar proportion of butyric acid was noted ($P<0.05$) in DF27 when compared to the other treatments. The ruminal concentration of NH_3-N showed some insignificant differences ($P>0.05$) among treatments. The daily FI was increased ($P<0.01$) in DF27 (1.131 g) when compared with DF0, while DF9 and DF18 showed intermediate consumption with no differences ($P>0.05$) among them. The ADG showed only difference (cubic effect, $P=0.02$) between DF9 and DF18. The highest feed conversion was observed (cubic effect, $P<0.01$) with DF18, with a value of 4.7 kg of feed to gain 1 kg of body weight. It could be concluded that the inclusion of up to 18 % of DF silage in the TMR of growing lamb diets, in hot regions of Mexico, may improve productive performance and ruminal fermentation kinetics, without any negative effects.

Keywords Average daily gain · Devil fish · Rumen fermentation · Silage

Abbreviations

ADG	Average daily gain
DF	Devil fish
FI	Feed intake
TMR	Total mixed ration
VFA	Volatile fatty acids

✉ Abdelfattah Zeidan Mohamed Salem
asalem70@yahoo.com

1 Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, P.O. 40660, Altamirano, México

2 Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de México, P.O. 50000, Toluca, México

3 Dairy Science Department, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt

4 North Florida Research and Education Center, Department of Animal Sciences, University of Florida, Marianna, FL, USA

Introduction

The *Plecostomus* spp., known locally in Guerrero state of Mexico as devil fish (DF, el pez diablo) or toad, is an invasive species (Loricariidae) that appeared in the freshwater bodies in Mexico in the 1990s and supported 46,000 people through fishing and processing jobs. Nowadays, it causes a serious problem for small fishermen in the coastal state of Guerrero and other parts of the Mexican country (Mendoza et al. 2009). The DF causes many fishing artifact damages and apparently compete with the commercial fish. Fishermen reject its use and exploitation for their own consumption or sale. As a direct result, this fish population has increased at the expense of commercially important fish such as tilapia, catfish, and other native fish. Ornelas et al. (2011) summarized major negative characteristics for DF including becoming a dominant fish species from México without natural enemies in the food chain. Moreover, this type of fish often ingests the eggs of other species that lay on the bottom of their habitat, thereby preventing the production of offspring.

This fish type (i.e., DF) has the potential to be used as high-protein supplements for farm animals. Ward et al. (1985) showed that ruminants can degrade the protein in fish silage after a period of initial adjustment. In general, the fish silage has been evaluated in hot regions of Mexico and other countries in feeding domestic animals, such as pigs, poultry, and cattle and even the fish themselves, with satisfactory results (Bertullo 1989; Al-Marzooqi et al. 2010; Ornelas et al. 2011). Ensiling can be an effective way for processing fish and using them as a feed ingredient (Al-Marzooqi et al. 2010). However, it has been difficult for small producers to use fish ensiling because of, among other issues, the difficulties of managing acidifying agents (acids) and the commercial implementation.

The silage preservation technique for feeds is based on fermentative acidification of substrates, which are subjected to the action of lactic acid-producing bacteria; the gradual accumulation decreases the pH of the medium and prevents the breakdown caused by organisms of putrefaction. However, a large portion of the silage crude protein is in the form of non-protein N, which is rapidly degraded in the rumen into ammonia (Ouellet et al., 1997). This process could cause dietary crude protein wastage once N requirements for microbial protein synthesis are met (Satter and Roffler 1975). Providing diets with ruminally protected or non-degradable amino acids like fish meal may enhance the growth of cattle fed silage due to increased amounts of amino acids available at the intestinal level (Owens and Zinn 1988). Fish silage production has an advantage where the proteolytic enzymes present in the fish hydrolyze the protein and fat causing an inhibition of spoilage and pathogenic-type bacteria, improving the product safety as animal feed (Al-Marzooqi et al. 2010). Fish silage can be mixed with cereals or other carbohydrate sources before feeding (Goddard and Perret 2005).

Technical conservation of fish and their by-products has been widely studied (Córdova et al. 1990; Pérez 1995; Cisneros et al. 2004). As a result, several techniques have been developed, such as mineral acid-treated silage (hydrochloric, sulfuric, and phosphoric acid), organic acids (acetic, formic), or a combination of them. In addition, a biological conservation technique by using substrates of vegetable origin (molasses, cassava flour) treated with fermentative bacteria (*Lactobacillus* and *Streptococcus*) or some fungi (Cisneros et al. 2004) were developed. Therefore, the objective of the current study was to evaluate the ruminal fermentation and production characteristics of the inclusion of DF silage in the diet of growing Criollo×Blackbelly lamb diets in hot region of Guerrero state of Mexico.

Materials and methods

Experimental location, animals, and treatments

The study was performed in the Sheep Production Unit and Animal Feed Science Laboratory of the Academic Unit of Veterinary Medicine at the Autonomous University of Guerrero, Guerrero, Mexico. Geographically, this place is located at 100°40′5″ west longitude and 18°24′30″ north latitude, with a rainfall of 1100 mm, an altitude of 240 m, a minimum temperature of 28 °C and a maximum of 43 °C (EMM 2001).

Twenty uncastrated Criollo×Blackbelly crossed lambs, a dual purpose sheep, especially for meat production, were used with an average live body weight of 18±1.2 kg, housed in pens of 4×4 m, and provided with feed and water bowls. Prior to the experimental period, the lambs were internal and externally dewormed with an oral product (based on 100 mg of closantel and 2 mg of ivermectin per milliliter) plus 0.5 mL of a multivitamin (containing 5,000,000 IU of vitamin A, 75, 000 IU of vitamin D, and 50 IU of vitamin E) applied intramuscularly.

Treatments consisted of total mixed ration (TMR) with four inclusion rates of DF silage (*Plecostomus* spp.) at 0 % inclusion (DF0), 9 % inclusion (DF9), 18 % inclusion (DF18), and 27 % inclusion (DF27). Lambs were assigned randomly in a completely randomized design into four treatments of five animals each. The TMR was provided during the experiment in two equal parts at 0800 and 1600 h, assigning 10 % more feed every 24 h, according to the tables of the NRC (2007).

The experiment lasted for 75 days including an adaptation period of 19 days. Data collection was repeated every 14 days through the experimental period. The complete diet was formulated with a computer software (Used Feed Formulation Done Again—UFFDA) (Pesti and Miller 1993). The TMR included ground oat hay (hammer mill Azteca 301021, Mexico), ground corn, soybean meal, ground cobs, and vitamins and minerals premix (Table 1). The diets were isonitrogenous and isocaloric (16.2 % crude protein and 3.6 Mcal metabolizable energy per kilogram; as supported by NRC 2007). The TMR used was designed for uncastrated male lamb with an initial average weight of 18–20 kg and an expected average daily gain (ADG) of 200 g daily.

Ruminal pH, volatile fatty acids, and ammonia-N

For determination the ruminal pH, 200 mL of ruminal liquid were taken from the ventral half of the rumen through a

Table 1 Ingredients and chemical composition (g/kg dry matter) of diets[a] fed to growing lambs with different percent of devil fish (*Plecostomus* spp.) silage in as fed basis

	DF0	DF9	DF18	DF27
Ingredients				
Oat straw	144.0	136.0	127.0	118.0
Milled corn	552.0	572.0	530.0	488.0
Soybean meal	180.0	142.0	107.0	72.0
Ground cob	49.0	46.3	42.6	40.0
Devil fish silage[b]	0.0	90.0	180.0	270.0
Calcium carbonate	8.5	8.4	8.4	7.2
Urea	4.5	4.2	3.9	3.6
Molasses	58.9	0.00	0.00	0.00
Vitamin A	3.1	1.1	1.1	1.2
Chemical composition				
Organic matter	963.0	960.0	951.0	940.0
Crude protein	162.0	162.0	162.0	162.0
Ether extract	33.0	35.0	36.0	37.0
Acid detergent fiber	103.0	100.0	96.0	91.0
Neutral detergent fiber	177.0	175.0	169.0	162.0

[a] Diets contained 0 (DF0), 9 (DF9), 18 (DF18), and 27 (DF27) % of devil fish from the total mixed ration

[b] The devil fish silage had a 4.2 pH, 31.9 % crude protein, and 2.8 % ether extract. Lactic acid not determined and a microbiological load in colony-forming units of aerobic mesophiles of 1 × 10 CFU/g

stomach tube, 4 h after providing the feed to each lamb of each treatment. The obtained rumen liquor was filtered through four layers of gauze to prevent excessive feed particles. The ruminal pH was measured with a digital potentiometer (Orion, Mexico). From the obtained filtered rumen fluid, about 6 mL were taken and placed in plastic vials and centrifuged at 14, 000× g for 10 min. Approximately 1.5 mL of the obtained supernatant was taken into 2.0-mL vials containing 0.35 mL of 25 % metaphosphoric acid for volatile fatty acid (VFA) determination.

The determination of total and individual VFA was achieved according to the technique of Erwin et al. (1961), in a gas chromatograph model Clarus 500 (Perkin Elmer®, USA), equipped with an Elite FFAP capillary column. Hydrogen was used as carrier gas at a flow of 5.5 mL/min with increased injector and detector temperature of 250 °C min to a final temperature of 140 °C, with a total run time of 8 min, as reported by Kung and Hession (1995). For the determination of ammonia-N, approximately 50 mL of the filtered rumen fluid were taken at each period, centrifuged at 10,000×g for 15 min. About 4.0 mL of the obtained supernatant was placed in 10-mL plastic vials containing 1.0 mL of 25 % metaphosphoric acid. Approximately 1.0 mL of this solution was placed in a 13×100-mm glass tube (Home Science Tools, MT, USA) containing 7.5 mL of phenol and 7.5 mL of sodium hypochlorite. The mixture was stirred in a vortex and incubated at 37 °C for 30 min. The absorbance reading was achieved in a spectrophotometer (UV Wavelength, Perkin Elmer®, USA) at a wavelength of 630 nm, as indicated by McCullough (1967).

Feed intake, average daily gain, and feed conversion

Voluntary feed intake (FI) in each group was estimated by the difference between feed offered and rejected every 24 h. To evaluate the ADG, each lamb was weighed in the morning before offering feeds with a digital scale (Torrey®, Mexico) on various dates: at the start of the experiment, at 19 days (which was the end of adaptation period), and then every 14 days. The ADG was calculated from the weight difference between two consecutive weighs. Feed conversion was calculated using the equation FI/ADG.

Statistical analysis

Data were analyzed as a completely randomized design, where lambs were the experimental units. Measured parameters (ruminal fermentation parameters, ADG, FI, and feed conversion) were analyzed using PROC MIXED of SAS (Ver.8.02, SAS Institute Inc., Cary, NC, USA) (SAS Institute Inc., 2001) of the following statistical model:

$$y_{ij} = \mu + d_i + \varepsilon_{ij}$$

where y_{ij} is the mean value measured on the jth lamb assigned to the ith diet, μ is the overall mean, d_i is the ith diet (DF silage levels), ε_{ij} is the random error associated with the jth lamb assigned to the ith diet. Duncan's test was used for the multiple comparisons of mean values. The linear, quadratic, or cubic effects of DF silage inclusion were determined using orthogonal polynomial contrasts in SAS, as defined by Steel and Torrie (1980).

Results

Rumen fermentation kinetics

The results of ruminal fermentation (Table 2) showed that the rumen pH values did not differ (P>0.05) among treatments. Total VFA concentrations and acetic acid molar proportion were not affected (P>0.05) by treatments. However, the molar proportion of propionic acid followed an irregular pattern as the control diet (DF0) was similar to DF18 and DF27. The diet that contained 9 % DF (i.e., DF9) showed the greatest concentration (linear effect P=0.03; quadratic effects P=0.05) of propionic acid compared to DF27. The molar proportion of butyric acid was greater (linear effect P<0.01; quadratic

Table 2 Effect of the ensiled devil fish (*Plecostomus* spp.—DF) inclusion in the diets[a] of growing lambs on ruminal fermentation parameters

	Diets				SEM	*P* value		
	DF0	DF9	DF18	DF27		Linear	Quadratic	Cubic
Ruminal pH	6.35	6.28	6.36	6.22	0.511	0.55	0.72	0.42
Total volatile fatty acids (mmol/L)	77.3	88.1	87.6	80.0	6.91	0.78	0.12	0.87
Acetic acid (mmol/100 mmol)	53.5	51.3	53.3	53.0	2.63	0.93	0.46	0.27
Propionic acid (mmol/100 mmol)	34.8	37.3	34.5	30.9	1.82	0.03	0.05	0.53
Butyric acid (mmol/100 mmol)	11.7	11.4	12.3	16.2	0.59	<0.01	0.05	0.06
Acetic/propionic ratio	1.6	1.5	1.6	1.9	0.61	0.06	0.07	0.81
Ammonia-N (mg/dL)	4.73	7.44	6.44	7.48	0.963	0.15	0.45	0.25

[a] Diets contained 0 (DF0), 9 (DF9), 18 (DF18), and 27 (DF27) % of devil fish from the total mixed ration

effects $P=0.05$) in the ruminal fluid of lambs fed diets containing 27 % of DF silage (i.e., DF27) compared with the other lambs. The concentration of ammonia-N showed no significant differences ($P>0.05$) among treatments.

Compared to those fed a diet free of DF (DF0), lambs fed diets containing DF at 27 % (i.e., DF27) had a higher ($P<0.01$) feed intake. However, there were no differences between DF9, DF18, and DF0. Lambs fed DF18 had the greatest ADG (cubic effect $P=0.02$) while the DF27 was similar to DF0 and DF9. Lambs of both DF27 and DF9 followed by DF0 had the least (cubic effects $P<0.01$) feed conversion compared with DF18 lambs. However, the differences were not significant between DF27, DF9, and DF0 (Table 3).

Discussion

Rumen fermentation kinetics

Results of ruminal pH showed that lambs fed different diets maintained the pH within the range of 6.22 to 6.36. This is a very important condition to maintain proper rumen function and activity of rumen microorganisms according to Ørscov (1989). He mentioned that values of ruminal pH below 6.2 can negatively affect the growth and activity of some bacteria, mainly by decreasing the availability of bicarbonate, leading to potential metabolic disorders as well as diarrhea. In the current study, the buffering capacity of VFA may have helped to maintain rumen pH stable. Ouellet et al. (1997) noted that rumen pH was not affected by feeding fish meal and fish protein hydrolysate throughout the 6-h post feeding sampling period compared to the control diet based on grass silage.

The lack of differences in the molar proportion of acetic acid was coinciding with that reported by Ouellet et al. (1997) who fed ruminally fistulated calves with diets based on fish meal and fish protein hydrolysate, comparing them with a control diet based on grass silage. Similarly, Abazinge et al. (1994) found equal proportions of acetic acid and propionic acid in lambs fed with crab waste silage and wheat forage compared to lambs fed a diet that included wheat forage silage without crab waste silage. Goodrich et al. (1984) stated that, for ruminant meat production, a lower proportion of acetic acid and a higher proportion of propionic acid is generally associated with improved efficiency in energy use and therefore better response in ADG. In contrary, Zavaleta (2010) explained that, if the animal is given forage finely chopped (as it was in the current experiment), the proportion of acetic acid is diminished and is compensated by an increase in propionic or butyric acid. Moore (1964) described that this is because the fragmentation of the feed aids digestion by increasing the surface area for bacterial enzymatic action. However, finely grinding the forage decreases its effective fiber action, as the transit through the rumen is faster. Marty and Preston (1970) explained that it is possible to increase the production of butyric acid to 25–35 % of total VFA, using

Table 3 Effect of inclusion the ensiled devil fish (*Plecostomus* spp.—DF) in the diets[a] of growing lambs on feed intake, average daily gain, and feed conversion

	Diet				SEM	*P* value		
	DF0	DF9	DF18	DF27		Linear	Quadratic	Cubic
Average daily gain (ADG g/day)	162.5	155.8	211.5	172.0	20.02	0.16	0.21	0.02
Feed intake (FI g/day)	879.3	995.8	991.7	1130.7	4.01	<0.01	0.78	0.14
Feed conversion (kg of FI/kg of ADG)	5.4	6.4	4.7	6.6	0.55	0.24	0.25	<0.01

[a] Diets contained 0 (DF0), 9 (DF9), 18 (DF18), and 27 (DF27) % of devil fish from the total mixed ration

diets with a high content of molasses and urea, which may explain why in the current study the lambs fed DF27 had higher proportion of butyric acid in their rumen since they received a greater proportion of DF silage which contained up to 70 % molasses. Cook and Miller (1965) disclosed that butyric acid is oxidized into acetyl radicals used in the Krebs cycle to produce energy and carbon dioxide. Hardwick et al. (1961) stated that the increase in the production of butyric in the rumen of dairy cows can increase milk fat content.

The results of ruminal ammonia-N concentrations indicated no significant differences in all experimental diets. Church and Pond (1992) mentioned that in the rumen, N sources derived from the degradation of dietary protein and non-protein nitrogen can be used by microorganisms for microbial protein synthesis. Forbes and France (1993) mentioned that the concentration of ammonia-N in ruminal fluid indicates the degree of degradation of the protein from feed in the rumen, suggesting that there is less amount of protein that escaped from the rumen and could be absorbed in the intestine. Slyter (1976) mentioned that feed proteins are degraded by microorganisms in the rumen into amino acids, ammonia and organic acids. Excessively low ammonia-N concentrations can cause a shortage of nitrogen for bacteria, reducing the digestibility of feed.

Feed intake, average daily gain, and feed conversion

The inclusion of 18 % DF silage in the diet of growing lambs increased ADG. This intermediate level of inclusion was likely the most profitable as it had the greatest feed conversion. These results differ from those of Viete and Bello (1988), who in their experiment, tested three different levels (100, 200, and 300 g/day) of fish silage as a dietary supplement in cattle, and noted that an intermediate level of supplementation with silage was not profitable. They referenced their results to the taste of silage, where animals do not consumed it, making the lowest percentage of inclusion as the most efficient in terms of conversion. Ornelas et al. (2011) noted that dry matter intake and weight gain were not different ($P>0.05$) when silage of DF was included at 0, 12, and 18 % in fattening beef cattle.

It is well known that the quality of meat depends mainly in the feeding strategies of animals especially for muscle tissue and fatty acids (Moreno-Indias et al. 2012). Meat attributes, including hardness, color, and flavor, depend on feed additives and fed diet (Moreno-Indias et al. 2012; Morales-de la Nuez et al. 2014). However, we did not study the meat quality characteristics of lambs or its sensory attributes. Many studies show that feeding fish silage did not affect the sensory attributes of the meat. The quality and flavor of the meat from animals fed fish silage has been reported to be acceptable (Tatterson and Windsor 2001). Al-Marzooqi et al. (2010) tested the inclusion of fish silage in broiler chickens (up to 20 % of soybean meal in broiler diets) and Pérez (1995) in pigs (up to 50 % of the protein supplied), obtaining normal carcass-quality measurements, while various organoleptic evaluations including odor, taste, juiciness, and texture were not affected by its inclusion.

Lambs fed DF27 had a higher FI and these results are in agreement with Lira (2010), who tested two experimental diets (with and without fish silage) in dairy cows, where a higher feed intake was observed with cows fed fish silage.

The best feed conversion was observed with DF18 lambs, which indicates that the lambs of this group, only need to consume 4.7 kg of feed to produce 1 kg of growth. According to these results, feed consumption was not a factor greatly influencing the average daily gain observed in lambs fed diet with 18 % silage. These results imply that the efficiency of use of feed consumed was better in DF18, as the lack of significant (although numerical) differences in FI and ADG between DF18 and 27, translated into significant difference in feed conversion for DF18, when compared to all other treatments. This improved efficiency can be explained by the response observed in the ruminal fermentation dynamics discussed subsequently.

Conclusion

Under conditions in which the experiment was conducted, the ruminal pH was within normal parameters, and showing a better response in the ruminal fermentation kinetics with a higher concentration of butyric acid and similar concentrations of acetic acid than the other experimental treatments. Ruminal ammonia-N concentration was observed below the normal range, indicating lower ruminal protein degradation. Including 18 % of DF silage in the diet of growing lambs, improved feed conversion relative to all other inclusion rates, without having a significant response in feed intake or ADG over the control treatment. Therefore, DF silage may be included at a rate of 18 % in the diet of growing lambs, with favorable results in productive performance and ruminal fermentation kinetics. Nevertheless, further studies should be carried out on aspects related to meat quality characteristics as well as a sensory analysis of these carcasses.

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Conflict of interest All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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