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Fatty acids stability in goat yoghurt

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ABSTRACT: Evaluation of fatty acids (FAs) stability in dairy products undergoing technological milk processing is important for subsequent determinations of nutritional value. The aim of the study was to assess FA composition in milk and its dairy product and to explore differences in the FA profile found in yoghurt compared to raw material (goat milk). In the present study, a reduced proportion of volatile FAs (VFA) that cause "goat flavor" was reported in goat yoghurt in comparison to the FA profile of milk. Conversely, an increase of medium-chain as well as beneficial long-chain and unsaturated FAs (UFA) was reported in yoghurt compared with milk. In all cases, the differences in the FA composition between milk and yoghurt were not significant; therefore, it was found that manufacturing of yoghurt had no major influence on FA composition.

Key words: goat, milk, fatty acids profile, yoghurt, fermentation.

Avaliação da estabilidade de ácidos graxos em iogurte de cabra

RESUMO: a avaliação da estabilidade de ácidos graxos (ags) em produtos lácteos submetidos ao processamento tecnológico de leite é importante para as determinações subsequentes do valor nutricional. O objetivo do estudo foi avaliar a composição de fa no leite e seus produtos lácteos e explorar as diferenças no perfil de fa encontradas no iogurte em comparação com a matéria-prima (leite de cabra). No presente estudo, uma proporção reduzida de ácidos graxos voláteis (agv) que causam "sabor de cabra" foi encontrada no iogurte de cabra em comparação com o perfil de fa do leite. Por outro lado, um aumento de ácidos graxos de cadeia longa e não saturados de cadeia média e benéfica (ufa) foi encontrado no iogurte em comparação com o leite. Em todos os casos, as diferenças na composição de fa entre leite e iogurte não foram significativas. Portanto, verificou-se que a fabricação de iogurte não teve grande influência na composição da fa.

Palavras-chave: cabra, leite, perfil de ácidos graxos, iogurte, fermentação.

INTRODUCTION

Goat milk is a high-value commodity in terms of nutrition because its composition is advantageous when compared to cow milk (PASTUSZKA et al., 2015). This nutritional advantage is related to its good digestibility and buffer capacity, differential composition of casein-fractions (HAM et al., 2010; WANG et al., 2017; BIADALA & KONIECZNY, 2018) and to more desirable fatty acid (FA) profile of goat milk fat (CHILLIARD et al., 2007; MARKIEWICZ-KESZYCKA et al., 2013; KALA et al., 2016). Milk fat, an important component of milk, influences chemical, sensory, and technological milk properties (CHILLIARD & FERLAY, 2004; MICHALSKI et al., 2004; BARŁOWSKA et al., 2011; SANT'ANA et al., 2013). In comparison to cow

milk, goat milk is characterized by higher proportion of short-chain FAs (12.60-19.44% vs. 6.19-11.38% (PASTUSZKA et al., 2015)), lower proportion of palmitic acid (16:0; 21.66-29.52% vs. 27.02-35.35%) and higher proportion of essential FAs – linoleic acid (18:2n-6; 1.60-2.38% vs. 1.33-2.04%) and α -linolenic acid (18:3n-3; 0.46-0.97% vs. 0.30-0.58% (KALA et al., 2016)), respectively.

These variances are caused by differences in FA biosynthesis among species (BERNARD et al., 2013), breeds or individuals (SORYAL et al., 2005; TALPUR et al., 2009); among others, due to the existence of genetic polymorphisms (MOIOLI et al., 2007; HANUS et al., 2018). Nevertheless, nutrition is undoubtedly considered as the most important factor influencing milk FA profile (CHILLIARD et al., 2007; TORAL et al., 2015). It must be emphasized

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that feeding strategy in goats is characterized predominantly by pasture (VAN NIEUWENHOVE et al., 2009; ALBENZIO et al., 2016) which differs depending on season, climate, area, botanical composition etc. (SZMATOLA et al., 2013; SALARI et al., 2016; PALMA et al., 2017; GUTIERREZ-PENA et al., 2018). Conjugated linoleic acid (CLA; 9c,11t-18:2) is nutritionally valuable FA and milk is its important source for humans (SERAFEIMIDOU et al., 2012). The CLA proportion is affected also by nutrition which can vary depending on other factors such as lactation stage, age, and breed (STRZAŁKOWSKA et al., 2009; TALPUR et al., 2009). In general, CLA content is higher in goat milk than in cow milk (BARŁOWSKA et al., 2011; TORAL et al., 2015).

The proportion of individual FAs in milk fat can vary during milk processing (MARTINI et al., 2016). Often, this is not a direct change in the proportion of FAs but rather it is a change in the molecular FA configuration, resulting in variations in the proportion of FA isomers. For example, the CLA content in milk and dairy products is stable but the method of milk treatment (e.g. pasteurization) and storage influence the proportion of CLA isomers and their intermediate products (JUNG & JUNG, 2002; YUE et al., 2016). Thus, the processing of milk has a substantial effect on the nutritional value of milk and dairy products (SLAČANAĆ et al., 2010; RAHMAWATI & SUNTORNSUK, 2016) and milk fat properties can also be affected (RAYNAL-LJUTOVAC et al., 2005; VAN NIEUWENHOVE et al., 2009). Furthermore, dairy products have different properties compared to raw material because chemical or microbial processes can positively affect their composition (MARTINI et al., 2016), for example, lower lactose content in cheeses (MCCARTHY et al., 2017), or a slightly higher calcium content in yoghurts (BILANDŽIĆ et al., 2015). Moreover, yoghurts have the beneficial properties due to probiotics (MAZOCHI et al., 2010).

According to the aforementioned reasons, evaluation of FA stability in milk and dairy products undergoing technological milk processing is important for subsequent determination of nutritional value. Until now, there have been only a few reports concerning this subject (CAIS-SOKOLIŃSKA et al., 2015; SUMARMONO et al., 2015; ZOIDIS et al., 2018). The recent studies were performed mainly on cow milk and its dairy products (BODKOWSKI et al., 2016; BERGAMASCHI & BITTANTE, 2017).

For this reason, the aim of the present study was to evaluate FA profile in yoghurt produced from goat milk and to assess the differences in FA profiles between milk and its product.

MATERIALS AND METHODS

The study was carried out in the Czech Republic at one goat farm (sea level 516 m, organic management system) over three sampling periods (May, August, and October). Goats were grazed on natural pasture with addition of feed mixture (1.6 kg/day; oats, barley, and sugar beet in ratio 1:1:0.5). Individual milk samples (n=40) were collected from Anglo-Nubian goats (n=12; 40% of primiparous, 60% of multiparous). Samples were immediately cooled (to 6 °C) and transported to the laboratory in a cool box. Each sample was divided into three parts: 1) 30 ml for determining milk composition and somatic cell count; 2) 30 ml for determining FA profile in milk fat; and 3) 100 ml for manufacturing into yoghurt.

Milk composition (Table 1) was determined by infrared spectroscopy using MilkoScan FT+ (Foss Electric, Germany) in accordance with valid standard

Table 1 - Mean, standard deviation (SD), minimum and maximum, and coefficient of variation (CV) of basic chemical contents including	g
somatic cell count (SCC) of Anglo-Nubian goat milk (n=40).	

Trait ^a	Mean	SD	Min.	Max.	CV ^b
Protein (%)	4.20	0.78	3.17	6.15	18.6
Casein (%)	3.30	0.72	2.34	5.04	21.9
Fat (%)	5.06	1.82	1.89	9.03	36.0
Lactose (%)	4.09	0.31	3.44	4.64	7.6
MSNF (%)	8.95	0.60	7.69	10.39	6.7
SCC (thousands·ml ⁻¹)	2 772	3 587	26	17 727	129
SCC (log)	3.06	0.68	1.41	4.25	22.3

[&]quot;MSNF: milk solids-not-fat; SCC: somatic cell count, log: logarithm log₁₀.

^bCV: coefficient of variation: (standard deviation/mean)·100.

(ČSN, 1999), and somatic cell count was determined by flow cytometry using Fossomatic 90 Instrument (Foss Electric, Germany) also in accordance with valid standard (ČSN, 1998).

For FA identification, milk fat was extracted with petroleum ether from freeze-dried milk samples. FAs in isolated fat were re-esterified to their methyl esters with a methanolic solution of potassium hydroxide. Methyl esters of FAs were determined by a gas chromatographic method using a Varian 3800 apparatus (Varian Techtron, USA) under conditions described in table 2. The identification of FAs in milk fat was carried out using analytical standards (Supelco, USA). Forty-nine FAs out of total 57 FAs observed in the chromatograms were identified. The proportions of individual FAs were calculated from the ratio of their peak area to the total peak area of all the observed acids – Figure 1.

Yoghurt was processed according to the conditions of the fermentation test – after milk pasteurization (85 °C for 5 minutes), 2 ml of Yo-Flex Harmony® culture were used per each 50 ml of milk (Chr. Hansen, Denmark) and the sample was incubated during 3.5 hours at 43 °C.

Obtained data were evaluated using Microsoft Office Excel 2010 and Statistica 9.1 (StatSoft CR). For statistical verification, one-way ANOVA, Student's *t*-test, correlation and linear regression analyses were used at usual levels of significance (0.05; 0.01; 0.001).

RESULTS AND DISCUSSION

There was a high proportion of saturated FAs (SFAs; 70.69%) in goat milk samples, consistent

with previous research (STRZAŁKOWSKA et al., 2009; MARKIEWICZ-KESZYCKA et al., 2013; SUMARMONO et al., 2015). A high SFA proportion is characteristic for ruminant milk, of which goat milk has the highest SFA proportion (HAENLEIN, 2004; SANT'ANA et al., 2013). The unsaturated FA (UFA) proportion for goat milk was 25.98%, with the majority being monounsaturated FAs (MUFAs; 21.92%). These values were also retained for the most part in yoghurt samples (Table 3). Differences in groups of FAs between raw material and manufactured yoghurt were statistically insignificant in all cases and the correlations for each group (between milk and yoghurt) were very high (r>0.91; *P*<0.001).

Changes in the FA profile of goat milk and the fermented products (kefir, yoghurt, yoghurt drink) manufactured from it were also found out (CAIS-SOKOLIŃSKA et al., 2015; SUMARMONO et al., 2015; BORKOVA et al., 2018). Moreover, the study on FA composition of kefir (CAIS-SOKOLIŃSKA et al., 2015) showed that it remains relatively stable during both the manufacturing and storage for 21 days (as compared to the FA composition of raw material).

Therefore, the FA composition of dairy products is not significantly affected during the manufacturing process. Despite the statistical insignificance found in our study, a number of changes that increased the nutritional value of the product were observed. For example, the SFA proportion slightly decreased and the UFA proportion increased in yoghurt compared to milk. Importantly, yoghurt had an increased MUFA and polyunsaturated FAs (PUFA) proportion compared to milk.

Table 2 - Parameters	of gas c	hromatographic	analysis a	of fatty acids.
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Parameter	Value
Column ^b	CP-Select CB for FAME ^c , 50 m x 0.25 mm, 0.25 μm thickness
Detector	Flame ionization detector
Temperature	
column	55 °C for 5 min; 40 °C/min up to 170 °C; 2.0 °C/min up to 196 °C; 10.0 °C/min up to 210 °C
injector	250 °C
detector	250 °C
Helium flow	1.8 ml/min
Injection	1 ml, split 10

^aVarian 3800 apparatus (Varian Techtron, USA); ^bVarian Inc. (USA); ^cFAME: fatty acid methyl esters.

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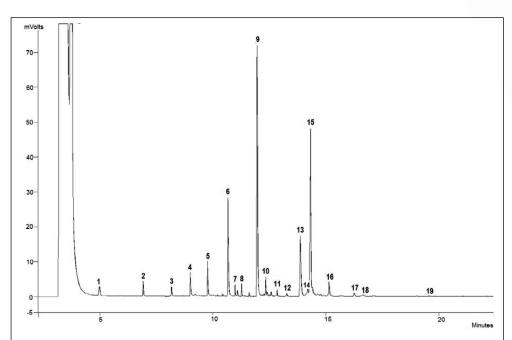


Figure 1 - Gas chromatogram of fatty acid methyl esters of milk fat; fatty acids^a were identified by analytical standards (Supelco, USA).

^a1: 4:0, butyric acid; 2: 6:0, caproic acid; 3: 8:0, caprylic acid; 4:10:0, capric acid; 5: 12:0, lauric acid; 6: 14:0, myristic acid; 7: 9c-14:1, myristoleic acid; 8: 15:0, pentadecanoic acid; 9: 16:0, palmitic acid; 10: 9c-16:1, palmitoleic acid; 11: 17:0, heptadecanoic acid; 12: 10c-17:1, heptadecenoic acid; 13: 18:0, stearic acid; 14: 11t-18:1, vaccenic acid; 15: 9c-18:1, oleic acid; 16: 18:2n-6, linoleic acid; 17: 18:3n-3, alpha linolenic acid; 18: 9c,11t-18:2, conjugated linoleic acid; 19: C20:4n-6, arachidonic acid.

In yoghurt, both the volatile FA proportion (VFA; 15.39% (milk) vs. 14.81% (yoghurt)) and short-chain FA proportion (SCFA; 21.61% (milk) vs. 21.03% (yoghurt)) decreased. This could be explained by the fermentation activity of lactic acid bacteria (JIA et al., 2016) or due to an effect of the fermentation process because of the volatile character of VFAs and SCFAs (BORKOVÁ et al., 2015; CAIS-SOKOLIŃSKA et al., 2015). The proportion of FA groups in milk corresponded with their proportion in yoghurt. Only small changes were noted in our research. These changes (Table 3) are expressed also as the percentage of increase/ decrease compared to proportion of FAs in milk. In this term, the highest difference was found out in VFAs (decrease by 3.75%).

The presence of VFAs in dairy products is beneficial because of their nutritional importance; therefore, their decrease is undesirable. Nevertheless, one advantage of this decrease in VFA values (with a simultaneous decrease in SCFA values) is a positive effect on the product's sensory properties because the FAs that cause "goat flavor" are included in these

groups. Therefore, it may be possible to remove an unfavorable "goat flavor" in final dairy products partly by decreasing the VFA and SCFA values (STRZAŁKOWSKA et al., 2009).

Some of 49 identified FAs were reported in very low concentrations. Herein, only those FAs with a proportion over 1% or those that showed marked changes were mentioned (Table 4). The FAs with the highest proportion in goat milk were, in order: 16:0 (26.96%), 9*c*-18:1 (18.33%), 14:0 (11.42%), 10:0 (10.19%), 18:0 (8.69%), and 12:0 (5.52%).

Similar proportions of these FAs were also reported in other studies (STRZAŁKOWSKA et al., 2009; SUMARMONO et al., 2015); however, a higher proportion of 10:0 (14.57%) and a lower proportion of 16:0 (21.65%) were found (STRZAŁKOWSKA et al., 2009). These differences could be the result of using milk from another goat breed (Polish White goats) or due to the different composition of feeding diet, as both these factors are known to influence the composition of milk fat (SZMATOLA et al., 2013; TORAL et al., 2015).

Table 3 - Mean, standard deviation (SD), minimum and maximum of selected groups of fatty acids (FA; % of total FAs) in raw material (Anglo-Nubian goat milk) and manufactured yoghurt samples, and relative percentage difference (RPD) and coefficient of correlation (r) between raw material (Anglo-Nubian goat milk) and manufactured yoghurt samples.

Groups ^a	Milk (n=40)				Yoghurt (n=40)				P^b	Relation between proportion of FA groups in milk and yoghurt	
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.		RPD (%)	r ^c
SFA	70.69	4.11	62.56	78.37	70.47	4.07	62.38	77.41	n.s.	-0.3	0.9398^{+++}
UFA	25.98	3.96	18.96	33.76	26.22	3.95	19.87	34.04	n.s.	+0.9	0.9388+++
MUFAcis	21.92	3.82	15.78	29.84	22.10	3.95	14.72	29.95	n.s.	+0.9	0.9303+++
PUFA	3.64	0.40	2.93	4.45	3.71	0.42	3.03	4.89	n.s.	+1.9	0.9386^{+++}
SCFA	21.61	2.30	17.27	26.94	21.03	2.20	16.60	25.34	n.s.	-2.7	0.9054+++
MCFA	44.23	2.70	39.32	51.63	44.65	2.71	39.51	51.49	n.s.	+0.9	0.9306+++
LCFA	34.15	3.47	27.72	42.88	34.32	3.68	27.81	43.37	n.s.	+0.5	0.9262+++
TFA	1.31	0.32	0.87	2.11	1.28	0.26	0.89	1.84	n.s.	-1.9	0.9479^{+++}
VFA	15.39	2.20	11.56	19.15	14.81	2.06	11.08	18.50	n.s.	-3.8	0.9430^{+++}
HFA	43.90	2.93	37.17	49.76	44.18	2.93	37.52	49.59	n.s.	+0.7	0.9492+++

^aSFA: saturated FAs; UFA: unsaturated FAs; MUFA *cis: cis* isomers of monounsaturated FAs; PUFA: polyunsaturated FAs (incl. conjugated linoleic acid); SCFA: short-chain FAs; MCFA: medium-chain FAs; LCFA: long-chain FAs; TFA: *trans* isomers of UFAs; VFA: volatile FAs; HFA: hypercholesterolemic FAs (C12:0 + C14:0 + C16:0);

Minor differences between FA profile in milk and yoghurt were observed also for individual FAs. These changes can be explained by enzymatic activities of lactic acid bacteria during the fermentation (BORKOVÁ et al., 2015; SUMARMONO et al., 2015). Noticeable differences included the 9*c*-14:1 proportion (0.47% (milk) *vs.* 0.50% (yoghurt) – Figure 2) and the 9*c*-16:1 proportion (0.66% (milk)

Table 4 - Mean, standard deviation (SD), minimum and maximum of selected individual fatty acids (FA; % of total FAs) in raw material (Anglo-Nubian goat milk) and manufactured yoghurt samples, and relative percentage difference (RPD) and coefficient of correlation (r) between raw material (Anglo-Nubian goat milk) and manufactured yoghurt samples.

FAs ^a		Milk (n=40)				Yoghurt (n=40)				Relation between proportion of individual FAs in milk and yoghurt	
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.		RPD (%)	r ^c
4:0	1.01	0.16	0.74	1.41	0.98	0.18	0.68	1.43	n.s.	-3.6	0.6152+++
6:0	1.66	0.25	1.21	2.13	1.61	0.25	1.21	2.12	n.s.	-3.0	0.8672^{+++}
8:0	2.52	0.41	1.87	3.35	2.42	0.39	1.79	3.23	n.s.	-4.2	0.9526+++
10:0	10.19	1.54	7.19	13.10	9.81	1.43	6.82	12.68	n.s.	-3.8	0.9632^{+++}
12:0	5.52	0.75	4.28	7.44	5.51	0.72	4.21	7.22	n.s.	<-0.1	0.8609^{+++}
14:0	11.42	1.13	9.28	14.09	11.45	1.10	9.39	13.96	n.s.	+0.2	0.9580^{+++}
9-c14:1	0.47	0.13	0.28	0.74	0.50	0.11	0.30	0.74	n.s.	+6.8	0.9833+++
15:0	1.05	0.20	0.71	1.91	1.06	0.19	0.75	1.88	n.s.	+1.0	0.9671***
16:0	26.96	2.40	22.88	31.67	27.22	2.27	23.34	31.46	n.s.	+1.0	0.9443+++
9-c16:1	0.66	0.18	0.39	1.07	0.72	0.16	0.46	1.11	n.s.	+8.7	0.9572^{+++}
18:0	8.69	1.49	5.57	11.86	8.72	1.37	5.62	11.49	n.s.	+0.4	0.9629^{+++}
9-c18:1	18.33	3.39	13.26	25.79	18.33	3.63	10.55	25.88	n.s.	0.0	0.9136+++
9 <i>c</i> ,11 <i>t</i> -18:2	0.53	0.19	0.26	0.94	0.54	0.19	0.29	1.00	n.s.	+2.5	0.9811***
18:2 <i>n-6</i>	1.98	0.32	1.45	2.78	2.03	0.32	1.54	2.87	n.s.	+2.3	0.9744+++
18:3 <i>n-3</i>	0.63	0.16	0.36	1.02	0.63	0.15	0.38	1.03	n.s.	0.4	0.9887***

^a9c,11t-18: conjugated linoleic acid (CLA);

^bP: significance level; n.s.: not significant;

^cr: +++; significance of correlation coefficient: *P*<0,001.

^bP: significance level; n.s.: not significant;

[°]r: +++; significance of correlation coefficient: P<0.001.

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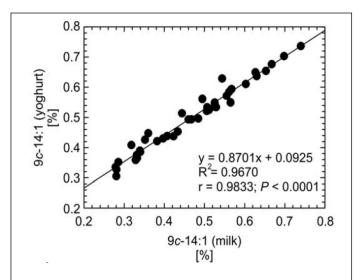


Figure 2 - Proportion of 9c-14:1 (myristoleic acid; % of total fatty acids) in yoghurt compared to goat milk samples (Anglo-Nubian, n=40) a .

 ${}^{a}R^{2}$: coefficient of determination; r: coefficient of correlation; P: significance level.

vs. 0.72% (yoghurt) – Figure 3). Conversely, the 9*c*-18:1 proportion remained stable (Figure 4). Except of 4:0 (r=0.6152; *P*<0.001), the correlation coefficients between milk and yoghurt were generally very

high (r>0.86; P<0.001) for all FAs. The FAs with a lower number of carbons, including those that cause the typical "goat flavor", experienced the greatest decrease, namely 6:0 (1.66% (milk) vs. 1.61%

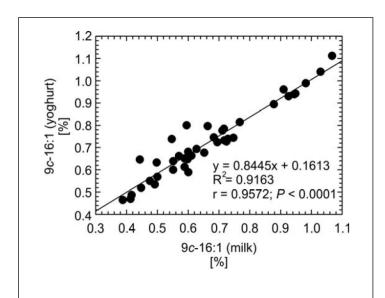


Figure 3 - Proportion of 9c-16:1 (palmitoleic acid; % of total fatty acids) in yoghurt compared to goat milk samples (Anglo-Nubian, n=40) a .

 ${}^{a}R^{2}$: coefficient of determination; r: coefficient of correlation; P: significance level.

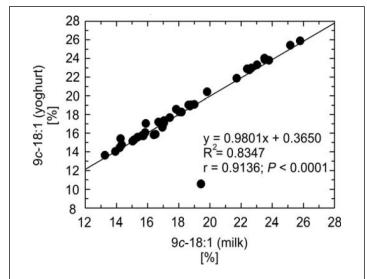


Figure 4 - Proportion of 9c-18:1 (oleic acid; % of total fatty acids) in yoghurt compared to goat milk samples (Anglo-Nubian, n=40).

 $^{a}R^{2}$: coefficient of determination; r: coefficient of correlation; P: significance level.

(yoghurt)), 8:0 (2.52% (milk) vs. 2.42% (yoghurt)), and 10:0 (10.19% (milk) vs. 9.81% (yoghurt)). The proportion of nearly all FAs with a higher number of carbons (between 12 and 22) increased. For example, the proportion of 16:0 was 26.96% in milk and 27.22% in yoghurt. However, it must be noted that the changes in FA proportions between milk and yoghurt were statistically insignificant in all cases. In conclusion, a modification of FA profile of raw milk seems to be an appropriate tool to the production of nutritionally beneficial goat dairy products.

CONCLUSION

Although, some changes in FA profile occurred during the processing of goat milk as a result of fermentation process, these changes can be characterized as minor. The results provided an information that the main role in the FA profile of final fermented dairy product plays the FA profile of used raw material. For these reasons, the greatest emphasis should be placed on the factors influencing the milk FA profile, particularly diet. Furthermore, the selection of goats with nutritionally more desirable milk fat composition could be another possibility to influence FA profile of final dairy products. The research focused on other fermented dairy products (like cheeses) would be helpful.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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