



# Comparison of Nutritive Values and *in vitro* Degradability of Steam Treated Rice Straw with Chinese Wild Rye and Alfalfa

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## ABSTRACT

The study was conducted to compare the chemical composition and *in vitro* degradability of steam treated rice straw, alfalfa hay (AH) and Chinese wild rye (CWR). The rice straws were treated with steam pressure, *i.e.* TRS-I (exposed to 15.5 kgf/cm<sup>2</sup> steam pressure for 90 sec), TRS-II (exposed to 15.5 kgf/cm<sup>2</sup> steam pressure for 120 sec). The results regarding chemical composition revealed that steam treated straw had higher ( $P<0.05$ ) dry matter (DM), ash and acid detergent fiber (ADF) contents; lower ( $P<0.05$ ) organic matter (OM) than AH and CWR, the ether extract (EE) were higher and neutral detergent fiber (NDF) were lower ( $P<0.05$ ) in AH and TRS-II, while the crude protein (CP) were higher and acid detergent lignin (ADL) were lower ( $P<0.05$ ) in AH than other groups. The concentration of acetate, butyrate and *iso*-butyrate were almost similar in TRS-II, AH and CWR; valeric and *iso*-valeric were lower ( $P<0.05$ ) in CWR and TRS-I than AH and TRS-II, while the propionate and total VFA were higher in AH but similar in CWR and treated rice straw. The *in vitro* degradability of DM, OM and NDF were significantly higher for AH and TRS-II while, ADF was higher for AH. Steam treated straws showed increased ( $P>0.05$ ) pH and NH<sub>3</sub>-N ( $P<0.05$ ), while total gas production was higher in AH and TRS-II than other groups. These findings suggest that steam treated straw had better nutrients values and degradability over Chinese wild rye, so it could be used as forage for ruminants.

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## Authors' Contribution

MN and TW conceived and designed the experiments. MN, NR and SA performed the experiments. MN and AK wrote the paper. DHK and MR checked and formatted the article.

## Key words

Microsatellite loci, Neosalanx Alfalfa, Chinese wild rye, Rice straw, Steam treatment.

## INTRODUCTION

Globally livestock species are reared to endeavor human demand of animal protein. Increasing human population and high demand of animal protein can only be overcome by increasing livestock production (Akinfemi *et al.*, 2010). Several roughages such as Chinese wild rye (*Leymus chinensis*) are widely used in ruminant's food to fulfill the demand, but due to low crude protein and energy contents, additional source of protein and energy is required to achieve the maximum animal production (Zhu *et al.*, 2013). While, establishing a good stand of high quality forages such as alfalfa (*Medicago sativa* L.) is challenging as the ideal alfalfa soil is deep and well-drained (Lacefield, 2013). Simultaneously, decreasing land for crop cultivation due to constrained by human population and urbanization the animal production is facing more challenges such as

increased production cost due to decreased availability of feed (Devendra and Leng, 2011; Trach, 1998). In this scenario, there is a widespread interest to search and or to utilize non-conventional feed ingredients for livestock.

On the other hand global paddy production forecast now stands at 730.2 million tons for the year 2013 (FAO, 2013). The average ratio of rice grain: rice husk:rice straw is 1: 0.25: 1.25, which reflects that there is an ample quantity of agricultural by-products such as straw of rice are available, out of which a huge quantity is burned in the field by the growers, causing serious pollution complications (Haefele *et al.*, 2011; Liu and Ørskov, 2000). Full use of crop residues as feeds for ruminant animals is of strategic importance in the developing countries where forage resources of good quality are in short supply. Therefore, effective utilization of the available feed resources crop residue based diets become more important and necessary step to alleviate the problem and it is suggested that attention must be shifted to the use of these available crop residues as a feed resource (Getinet and Yoseph, 2014; Devendra and Leng, 2011).

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As rice straw is poorly fermented, it has lower rates of disappearance in the rumen (Sarnklong *et al.*, 2010) therefore, feeding only rice straw does not provide enough nutrients to the ruminants to maintain high production levels due to the low nutritive value of this highly lignified material. This issue needs an attention to develop an efficient method for hydrolyzing fibrous materials in rice straw (Liu and Ørskov, 2000) and to utilize it as livestock feed. Steam explosion treatment has shown significant potential as a cost-effective pre-treatment of lignocellulosics (Castro, 1994). It has been shown to increase energy availability by solubilization of cellulose and hemicellulose and/or by freeing digestible materials from lignin or silica (Liu and Ørskov, 2000).

To the best of our knowledge, almost no study has been conducted to compare steam treated rice straw with Chinese wild rye grass or alfalfa hay as an additional forage source for ruminants. Hence, the aim of this study was to evaluate the effectiveness of the steam explosion treatment to enhance nutritive value of rice straw and its use as an unconventional feed source.

## MATERIALS AND METHODS

### *Samples preparation and steam explosion treatments*

Rice straw was cut into 3-5 cm length and treated with steam at steam pressures 15.5 kgf/cm<sup>2</sup> for 90 seconds (TRS-I) and 120 seconds (TRS-II), respectively, by using a steam explosion machine, dried and stored according to the method described by Li *et al.* (2013) for further analysis. Chinese wild rye and alfalfa samples were also prepared in accordance with the experimental design.

### *Chemical analysis of sample grasses*

Samples were forced air dried in oven at 65°C for 48 h for determination of dry matter (DM), organic matter (OM) and ash contents of the samples were determined according to the methods described by AOAC (2000). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by using an Ankom 200 fiber analyzer following the user's instructions (Ankom Technology, 2011). Acid detergent lignin (ADL) contents were determined by using acid detergent lignin beakers method (Ankom Technology, 2005). The ether extract (EE) of samples was extracted with methanol by using soxhlet apparatus. Crude protein (CP) was determined by Kjeldahl method with Kjeltec 2300 apparatus of Foss USA.

### *In vitro fermentation*

The rumen fluid was collected before the morning feeding from six ruminally-fistulated Xuhua goats under a continuous flow of CO<sub>2</sub> thoroughly mixed and strained

through four layers of muslin cloth into a pre-warmed insulated thermos. The donor goats were offered diet with an equal ratio of forage and concentrate (Table I). The diet was provided twice a day at 9:00 am and 05:00 pm, the fresh water was made available 24 h.

**Table I.- Ingredient (g/kg) and chemical composition (%) of donor goats diet.**

Item	
<b>Ingredients (g/kg)</b>	
Maize	365.25
Soya Bean	105.35
Stone powder	7.15
Ca (HPO <sub>4</sub> ) <sub>2</sub>	11.13
Salt	5.00
Premix	6.12
Rice Straw	500.00
<b>Chemical composition of feed (%)</b>	
Crude Protein	12.50
NDF	43.80
Ca	0.73
P	0.30

NDF, neutral detergent fiber

*In vitro* degradability of DM, OM, NDF, ADF and gas production (IVGP) were determined according to Tilley and Terry (1963). The grass samples (2.5 ± 0.1 g) were weighed and put into *in vitro* fermentation bottles. The bottles were filled with 50 ml of medium consisting of 1:4 ratios of rumen fluid and artificial saliva, which was prepared according to the McDougall (1948). The bottles were sealed with a rubber stopper equipped with Bunsen valve for gas escape, all bottles were incubated at 39°C up to 48 h. At 0, 2, 4, 8, 12, 24, 36 and 48 h of incubation, five bottles from each treatment were collected and unsealed; gas volume was measured by reading and recording the amount of gas volume after incubation by using a 100 ml glass syringe connected to the incubation bottle with a needle, pH and gas volume were recorded immediately. The fluid collected by filtering through nylon bags into tubes and stored at -20°C for volatile fatty acids (VFAs) and NH<sub>3</sub>-N analysis. The sample residues in the bags were flushed with tap water until it became colorless, dried at 65°C for 24 h and stored for further analysis.

### *NH<sub>3</sub>-N determination*

After centrifugation of fermented samples; 1 ml supernatant was added to 9 ml distilled water, thoroughly mixed one millilitre of this mixture added to 0.04 ml of Nessler reagent and 3.6 ml of distilled water. In order to know the linear relationship between varying concentration

of  $\text{NH}_4\text{Cl}$  stander solution and color intensity produced by nesslerization a stander curve was made by using tubes of different concentrations of stander solution as described by Ali *et al.* (2008), the color intensity was measured at 540 nm after setting it at 0 absorbance with blank. The  $\text{NH}_3\text{-N}$  concentration was calculated as below:

$$\text{NH}_3\text{-N} \left( \frac{\text{mg}}{100\text{ml}} \right) = \frac{\text{Absorbance of samples}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

Gas chromatography (Agilent Technologies 7890A, GC system) was used to determine acetate, propionate, butyrate, *iso*-butyrate, valeric and *iso*-valeric concentrations as per the method of Bjorkman and Forslund (1986), with some modification. A 30 m×0.25 mm i.d. fused silica capillary column with a 0.25  $\mu\text{m}$  film was used (Catalog No: 24107, Supelco). The nitrogen, hydrogen and air were used as a carrier, fuel combustion-supporting gases, respectively. The temperature of the column, injector and the flame ionization detector were 120 °C, 180 °C and 210 °C, respectively.

**Table II.- Chemical composition (g/kg DM) of alfalfa hay, Chinese wild rye and steam treated rice straws.**

Item	Treatment			
	AH	CWR	TRS-I	TRS-II
DM (g/kg)	893.4±10.2 <sup>c</sup>	905.2±12.5 <sup>b</sup>	911.9±11.1 <sup>b</sup>	921.6±12.2 <sup>a</sup>
OM	932.9±10.9 <sup>a</sup>	945.6±15.8 <sup>a</sup>	897.8±17.5 <sup>b</sup>	864.1±15.3 <sup>b</sup>
Ash	67.1±1.9 <sup>b</sup>	54.4±3.8 <sup>b</sup>	102.2±4.6 <sup>a</sup>	135.9±5.6 <sup>a</sup>
EE	22.6±1.7 <sup>c</sup>	34.47±1.1 <sup>a</sup>	28.8±1.0 <sup>b</sup>	35.8±1.3 <sup>a</sup>
CP	144.6±8.1 <sup>a</sup>	44.9±1.1 <sup>c</sup>	53.55±2.1 <sup>b</sup>	54.2±2.5 <sup>b</sup>
NDF	605.8±13.4 <sup>b</sup>	757.2±23.2 <sup>a</sup>	783.2±17.2 <sup>a</sup>	671.7±19.6 <sup>b</sup>
ADF	375.6±12.6 <sup>b</sup>	415.9±13.1 <sup>b</sup>	517.8±18.9 <sup>a</sup>	461.6±18.0 <sup>a</sup>
ADL	58.3±2.4 <sup>b</sup>	63.7±3.1 <sup>a</sup>	64.3±2.2 <sup>a</sup>	61.8±4.1 <sup>a</sup>

AH, alfalfa hay; CWR, Chinese wild rye; TRS-I, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 90 sec; TRS-II, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 120 sec. DM, dry matter; OM, organic matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin. <sup>abc</sup> Mean values in the same row that do not share a common letter are significantly different (P<0.05); n = 5. Values are presented as mean ±SE.

### Statistical analysis

The data were analyzed by ONE-way ANOVA (SAS 9.0, SAS Institute Inc., Cary, NC). Five replicates were used for determination of chemical composition and other parameters of *in vitro* degradability and results were presented as mean ± SEM. Significant differences among means were evaluated using Tukey's comparison test at (P<0.05).

## RESULTS

### Chemical composition

Results regarding chemical composition of rice straw

treated with steam (TRS-I and TRS-II), Chinese wild rye (*Leymus chinensis*) (CWR) and alfalfa hay (AH) are presented in Table II. DM were higher (P<0.05) for TRS-II than that of AH, CWR and TRS-I. While higher (P<0.05) organic matter (OM) for AH and CWR was recorded than that of treated rice straw (TRS-I and TRS-II). The ash and ADF contents were significantly high TRS-II and TRS-I, as compared to AH and CWR. Crude protein (CP) contents were significantly higher and ADL were lower for AH than that of CWR, TRS-I and TRS-II. Ether Extract (EE) was significantly higher for the CWR and TRS-II as compare with AH and TRS-I. Significantly low NDF contents for AH and for TRS-II were recorded than that of CWR and TRS-I.

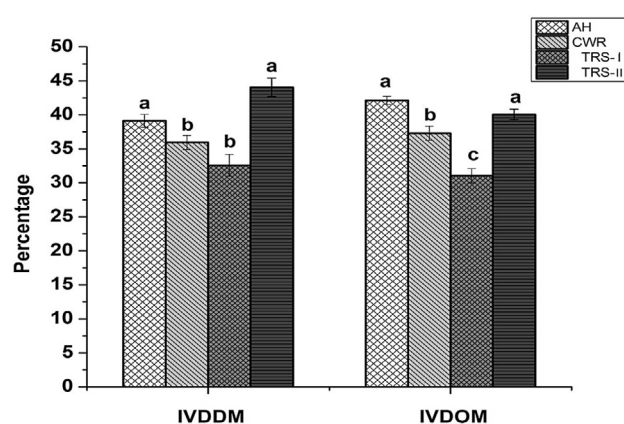


Fig. 1. *In vitro* dry matter digestibility (IVDDM %) and organic matter digestibility (IVDOM %) of steam treated rice straws, alfalfa hay and Chinese wild rye. AH, alfalfa hay; CWR, Chinese wild rye; TRS-I, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 90 sec; TRS-II, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 120 sec. <sup>abc</sup> Mean values that do not share a common letter are significantly different (P<0.05); n = 5. Values are presented as mean ±SE.

### In vitro degradability

The *in vitro* dry matter degradability (IVDMD) was higher by 8.90% and 20.11% for AH and 22.57% and 35.19% for TRS-II as compared with CWR and TRS-I, respectively. Similarly the *in vitro* organic matter degradability (IVDOM) was significantly higher by 13.01% and 35.77% and 7.42% and 29.05% for AH and TRS-II than that of CWR and TRS-I, respectively (Fig. 1). The DNDF was higher (P<0.05) by 21.52% and 21.15% in AH and 12.17% and 11.84% in TRS-II than CWR and TRS-I, respectively. Whereas, DADF were significantly higher in AH by 32.20%, 87.01% and 16.06% than that of CWR, TRS-I and TRS-II, respectively (Fig. 2).

The results regarding cumulative gas production, pH and  $\text{NH}_3\text{-N}$  are presented in Figure 3, gas production was

significantly higher for TRS-II and AH, than that of TRS-I and CWR, respectively.  $\text{NH}_3\text{-N}$  was lower ( $P<0.05$ ) in AH as compared with CWR, TRS-I and TRS-II, respectively. The pH was not differ ( $P>0.05$ ) among the treated straw and other groups.

#### Volatile fatty acids

Individual and total VFA concentrations are presented in Table III. Higher ( $P<0.05$ ) total volatile AH, alfalfa hay; CWR, Chinese wild rye; TRS-I, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 90 sec; TRS-II, rice straw treated with steam explosion at 15.5 kgf/cm<sup>2</sup> for 120 sec. <sup>abc</sup> Mean values in the same row that do not share a common letter are significantly different ( $P<0.05$ );  $n = 5$ . Values are presented as mean  $\pm$ SE. fatty acids (TVFAs) concentration was recorded for AH group followed by TRS-II, CWR and TRS-I, TVFAs were 9.15%, 13.48% and 23.71% higher in AH than that of TRS-II, CWR and TRS-I, respectively. Similarly propionate concentration was higher ( $P<0.05$ ) by 11.04%, 18.47% and 20.43% while, valeric acid was higher by 39.09%, 73.86% and 109.59% and *iso*-valeric acid was higher by 36.90%, 161.36% and 202.63% in AH as compared with TRS-II, CWR and TRS-I, respectively. Steam treated straws had lower ( $P<0.05$ ) butyrate concentration by 38.06% and 26.00% for TRS-I and 27.68% and 24.98% for TRS-II as compared with AH and CWR, respectively. The concentrations of acetate and *iso*-butyrate were significantly higher by 10.45% and 20.56% for AH and 5.50% and 15.16% for TRS-II, than that of CWR and TRS-I, respectively.

## DISCUSSION

Steam and pressure treatments are known to disrupt lignocellulosics, which improved utilization of cell wall polysaccharides by cell-free enzymes and rumen microbes (Castro and Machado, 1990). Lignin transformation and hemicellulose solubilisation to free the carbohydrates from

the lignin and by breaking the chemical and physical bonds are the main advantages of steam explosion treatment (Viola *et al.*, 2008; Brodeur *et al.*, 2011). The extent of cell wall solubility of steam treated straw was found to positively

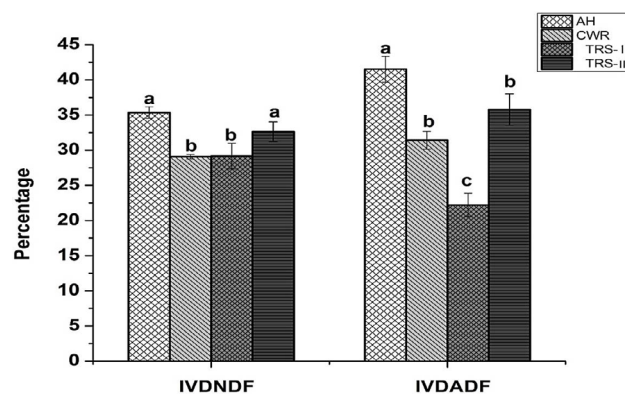


Fig. 2. *In vitro* DADF (%) and DADF (%) steam treated rice straws, alfalfa hay and Chinese wild rye. For abbreviations and statistical details, see Figure 1.

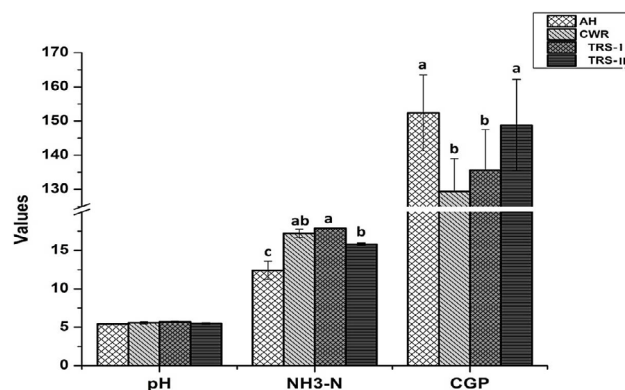


Fig. 3. *In vitro* cumulative gas production (CGP), pH and  $\text{NH}_3\text{-N}$  (mg/100ml) concentrations of steam treated rice straws, alfalfa hay and Chinese wild rye. For abbreviations and statistical details, see Figure 1

Table III.- *In vitro* volatile fatty acid production (mmol/l) of alfalfa hay, Chinese wild rye and steam treated rice straws.

Item	Treatment			
	AH	CWR	TRS-I	TRS-II
Acetate	79.29 $\pm$ 3.52 <sup>a</sup>	71.79 $\pm$ 2.83 <sup>b</sup>	65.77 $\pm$ 2.57 <sup>c</sup>	75.74 $\pm$ 3.67 <sup>ab</sup>
Propionate	47.28 $\pm$ 1.97 <sup>a</sup>	39.91 $\pm$ 1.14 <sup>b</sup>	39.26 $\pm$ 1.89 <sup>b</sup>	42.58 $\pm$ 1.25 <sup>b</sup>
Butyrate	13.24 $\pm$ 0.42 <sup>a</sup>	12.96 $\pm$ 0.61 <sup>a</sup>	9.59 $\pm$ 0.39 <sup>b</sup>	10.37 $\pm$ 0.45 <sup>ab</sup>
Iso-butyrate	2.39 $\pm$ 0.05 <sup>a</sup>	1.68 $\pm$ 0.09 <sup>b</sup>	1.36 $\pm$ 0.07 <sup>c</sup>	2.08 $\pm$ 0.08 <sup>ab</sup>
Valeric acid	1.53 $\pm$ 0.10 <sup>a</sup>	0.88 $\pm$ 0.03 <sup>c</sup>	0.73 $\pm$ 0.05 <sup>c</sup>	1.10 $\pm$ 0.02 <sup>b</sup>
Iso-valeric	1.15 $\pm$ 0.05 <sup>a</sup>	0.44 $\pm$ 0.03 <sup>c</sup>	0.38 $\pm$ 0.02 <sup>c</sup>	0.84 $\pm$ 0.06 <sup>b</sup>
Total VFA	144.87 $\pm$ 5.24 <sup>a</sup>	127.66 $\pm$ 3.56 <sup>b</sup>	117.10 $\pm$ 4.75 <sup>c</sup>	132.72 $\pm$ 4.49 <sup>b</sup>

For abbreviations, see Table II.  
VFA, volatile fatty acid



respond to the pressure of steam conditions (Kim *et al.*, 2005). The lower OM contents were observed in steam treated rice straws than that of Alfalfa hay (AH) and Chinese wild rye (CWR), these results are in agreement with Kim *et al.* (2005) who reported that OM values were decreased with increasing of pressure at the same time. While, Chaji *et al.* (2010) reported a declining trend for NDF content and a rising trend for ADF contents with the increasing steam pressure and time of treatment. In addition steam explosion process increased the susceptibility of cellulose to enzymatic hydrolysis and cellulose will be more accessible for rumen microbial enzymes, similarly we observed that the steam treated rice straw with 15.5 kgf/cm<sup>2</sup> for 120 sec, (TRS-II) had lower, but almost similar NDF as Alfalfa hay (AH) while, at the same pressure with lower time (15.5 kgf/cm<sup>2</sup> for 90 sec) the NDF contents were higher than TRS-II and AH but similar as found in Chinese wild rye (CWR). However, the ADF contents were higher in both steam treated straws as compared to AH and CWR. NH<sub>3</sub>-N concentration of fermented liquid was similar in TRS-II and AH, but lower than other treatments, enhanced utilization of NH<sub>3</sub>-N by rumen bacteria suggest that amino acid from protein were less available for deamination into microbial cell (McMahon *et al.*, 1999). Cumulative gas production was higher in AH and TRS-II than CWR and TRS-I, researchers have reported that application of steam treatment increased the gas production parameters (Chaji *et al.*, 2010) similar trend was also recorded in our previous work (Naeem *et al.*, 2014) that steam treated straw had higher cumulative gas production as compared to the untreated one.

The *in vitro* dry matter (DM), organic matter (OM) and NDF degradability for TRS-II and AH were similar but higher than TRS-I and CWR, which is in agreement of other work who reported that AH had higher degradation than CWR (Zhu *et al.*, 2013) and steam treatment of rice straw at 15.5 kgf/cm<sup>2</sup> for 120 sec may cause partial or complete hydrolysis of hemicellulose fractions into more soluble components hence increased the digestibility (Toussaint *et al.*, 1991). While the *in vitro* ADF degradability of treated straws was lower than AH and CWR which may be a cause of lignin chemical complex formed with cellulose (Han, 1975). Kim *et al.* (2005) reported higher concentrations of VFAs in rice treated with 16 kgf/cm<sup>2</sup> likewise we observed that the fermented liquid concentration of acetate, propionate, butyrate, *iso*-butyrate, valeric, *iso*-valeric concentrations and total volatile fatty acids (TVFAs) were higher in AH followed by TRS-II and CWR, respectively. Although the nutritive value of treated straw in this study was found lower than alfalfa hay but there was significant variation in chemical composition and degradability of steam treated straw at 15.5 kgf/cm<sup>2</sup> for 120 sec (TRS-II) over CWR, similar finding were reported by Liu *et*

*al.* (1999) and FAO (1998) that the digestibility of steam treated straw was comparable to the hay of medium quality.

Although the nutritive value of treated straw was lower than alfalfa hay, but rice straw treated with steam at 15.5 kgf/cm<sup>2</sup> for 120, had better nutrient values and digestibility over Chinese wild rye (*Leymus chinensis*), so it could be used as a forage source for ruminant nutrition.

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### Statement of conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article

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