

Spineless cactus-based diets associated with various nitrogen sources in sheep diets

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Abstract

This study evaluated the effects of various nitrogen (N) sources augmenting spineless cactus-based diets on intake, digestibility, rumen kinetics and N balance in sheep. Eight rumen-fistulated sheep with an average initial bodyweight of 62 ± 6.83 kg were allotted to one of two 4 x 4 Latin squares. The diets consisted of spineless cactus (*Nopalea cochenillifera*), Tifton hay, corn grain, vegetable oil and a mineral mixture augmented with either soybean meal (SBM), cottonseed meal (CSM), whole cottonseed (WCS) or urea (U) to provide additional nitrogen. Dry matter (DM), crude protein (CP), and neutral detergent fibre (NDF) intakes were similar ($P > 0.05$) for sheep fed the diets with SBM, CSM, and WCS. The digestibility coefficients of DM and CP were similar ($P > 0.05$) for SM, CM, and U diets. The rate of DM disappearance from the rumen did not differ ($P > 0.05$) between treatments. However, the rumen ammonia, pH and N balance was higher ($P < 0.05$) for sheep fed the WCS and U diets. The N absorbed and N retained did not differ ($P > 0.05$) between the diets. Soybean meal and CSM are similar in their effects and can be used for sheep in diets based on spineless cactus. Whole cottonseed and U are interesting sources of N but should be used with caution owing to their effects on digestibility and nutrient intake in sheep.

Keywords: ammonia nitrogen, cottonseed meal, *Nopalea*, rumen pH, urea

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Introduction

Sheep meat production is an important economic activity in tropical and subtropical areas of the planet. In these regions the seasonality of rainfall causes variations in the nutritional regime. Thus, supplementation during the dry season provides a means of maintaining the nutritional and productive status of the herd (Ben Salem & Smith, 2008).

High-protein concentrates, including the grains of legume plants, oilseeds, and meals and cakes that are made from their by-products, are generally used in the supplementation of sheep. However, the types of grain and by-product that are used most frequently in animal feed vary with location. In Brazil, SBM and by-products of cotton production, such as seed and meal, are the most frequently used protein supplements to ruminant rations (Pinto & Millen, 2019). Although SBM and CSM are excellent protein sources for ruminant diets, they differ in protein quality and ruminal degradation (Marcondes *et al.*, 2009; Queiroz *et al.*, 2010). The cost of these feedstuffs is an important limitation for farmers who are considering their use to supplement the diets of sheep during the dry season. For this reason, less expensive ingredients such as urea can be used strategically in supplementing sheep in tropical areas (Patra, 2015).

The source of dietary energy that is used in association with the protein concentrate is the most important factor determining the efficiency of microbial protein synthesis in the rumen (Galyean & Tedeschi, 2014). In this context, the energy derived from fermentation of starch or pectin in association with N sources influences several ruminal characteristics (Santos *et al.*, 2020). A promising source of pectin for tropical

areas is spineless cactus, which is rich in non-fibrous carbohydrates [588 g/kg DM] (Santos *et al.*, 2018; Siqueira *et al.*, 2019). This Cactaceae can be included at rates up to 500 g/kg of DM in diets for sheep to optimise the use of nutrients, including protein (Maciel *et al.*, 2019).

It was hypothesised that altering the protein source will change N metabolism in sheep fed spineless cactus-based diets. Thus, the objective was to evaluate the effects of various N sources in association with spineless cactus on intake, digestibility, ruminal parameters, and N balance in sheep.

Material and methods

All the procedures used in this study were authorised by the Ethics Committee on Animal Use, CEUA/UFRPE (licence 053/2015). The experiment was carried out in Pernambuco, Brazil (8°04'03" S and 34°55'00" W), at an altitude of 4 metres. According to Köppen classification system, the climate is of the AMS-type, which is hot and humid, with an average annual temperature of 25.2 C.

Eight castrated male Santa Inês sheep that had been fitted with rumen fistulas were treated against ecto- and endo-parasites and housed in individual pens (2.00 x 1.80 m). The sheep had an average weight of 62 ± 6.83 kg. The pens were equipped with individual feeders and drinkers. An adaptation period of 14 days allowed the sheep to become accustomed to the facilities and handling. After that, the animals were allotted to one of two 4 x 4 Latin squares. The trial lasted 92 days, with four consecutive 23-day periods, with each period being divided into 16 days for adaptation to diets and seven days for sampling.

The experimental diets, which were formulated according to NRC (2007), consisted of spineless cactus (*Nopalea cochenillifera*), Tifton 85 hay (*Cynodon* spp.), corn grain, vegetable oil and a mineral mixture with SBM, cottonseed meal (CSM), whole cottonseed (WCS) or urea being provided as a source of supplemental N. The nutritional composition of the ingredients is shown in Table 1. Fresh spineless cactus was processed in a shredder and offered immediately. The Tifton hay was ground through a 13 mm sieve. The corn grain, SBM and CSM were ground through a 4.5 mm sieve. The WCS was supplied whole. Diets (Table 2) were offered ad libitum as mixed rations at 8h00 (60%), and 16h00 (40%), with the amount offered being adjusted daily according to the previous day's intake to allow for 10% orts. Voluntary intake of dry matter and nutrients was calculated by the difference between the amounts of feed that were offered and orts from the previous day. For the apparent digestibility assay (17th to 19th day of each period) faeces were collected from each animal. At the end of each collection day, the faeces were weighed and homogenised, and a 10% aliquot was taken. The ingredients offered and orts were sampled over the same period. All the samples were pooled per animal and period.

Table 1 Chemical composition of feed ingredients fed to sheep in evaluating alternative sources of nitrogen

	Spineless cactus	Corn grain	Tifton 85 hay	Soybean meal	Cottonseed meal	Whole cottonseed
Dry matter, g/kg as fed	83.90	886.0	908.8	894.10	897.10	915.69
Organic matter, g/kg DM	881.21	985.60	919.90	937.20	945.90	963.30
Crude protein, g/kg DM	56.10	90.20	74.60	488.15	451.72	233.64
Ether extract, g/kg DM	5.10	61.30	8.20	31.20	22.70	191.20
NDFap, g/kg DM	310.86	112.28	799.98	139.10	228.84	452.94
ADF, g/kg DM	136.45	26.96	413.65	68.30	199.64	325.92
NFC, g/kg DM	509.24	721.82	37.12	278.95	242.64	85.62

DM: dry matter, NDFap: neutral detergent fibre corrected for ash and protein, ADF: acid detergent fibre, NFC: non-fibrous carbohydrate

Table 2 Quantity of ingredients and chemical composition of experimental diets fed to sheep in evaluating alternative sources of nitrogen

Ingredients (g/kg DM)	Nitrogen sources			
	Soybean meal	Cottonseed meal	Whole cottonseed	Urea
Spineless cactus	470.0	450.0	400.0	410.0
Tifton 85 hay	300.0	260.0	224.0	280.0
Corn grain	-	60.0	80.0	254.0
Soybean meal	210.0	-	-	-
Cottonseed meal	-	210.0	-	-
Whole cottonseed	-	-	270.0	-
Urea	-	-	16.0	26.0
Oil vegetable	10.0	10.0	-	20.0
Mineral mixture ¹	10.0	10.0	10.0	10.0
Chemical composition				
Dry matter, g/kg as fed	161.62	167.45	184.86	181.42
Organic matter, g/kg DM	890.60	897.14	897.48	876.51
Crude protein, g/kg DM	151.26	144.91	154.57	140.13
Ether extract, g/kg DM	11.85	13.26	60.64	20.37
NDFap, g/kg DM	416.06	403.43	435.57	380.72
ADF, g/kg DM	202.57	212.49	237.39	178.61
NFC, g/kg DM	297.09	318.97	261.92	374.03
Total digestible nutrients, %	69.50	69.00	70.01	71.7

DM: dry matter, NDFap: neutral detergent fibre corrected for ash and protein, ADF: acid detergent fibre, NFC: non-fibrous carbohydrate

¹Vitamin A: 50,000 IU, vitamin D3: 6,000 IU, vitamin E: 300 mg, calcium: 115 g, phosphorus (solubility in citric acid 2%: 90.0%): 56 g, magnesium: 15 g, sulfur: 28 g, sodium: 98 g, iron: 1,000 mg, copper: 100 mg, manganese: 1,440 mg, zinc: 1320 mg, selenium: 24 mg, iodine: 8 mg, cobalt: 8 mg, fluorine: 933 mg, per kg

Feeds, Orts, and faeces were oven-dried at 55 °C for 72 hours and processed through a Willey mill using 2 mm and 1 mm sieves. Dry matter (method 934.01), ash (method 942.05), crude protein (CP) (method 968.06) and ether extract (EE) (method 920.39) were determined according to AOAC (2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as described by Van Soest (1991), and correction of NDF for ash and protein (NDFap) followed the methodology described by Licitra *et al.* (1996) and Mertens (2002). Non-fibrous carbohydrates (NFC) were quantified according to Hall (2000). Total digestible nutrients (TDN) were calculated according to Weiss (1999).

Samples of the rumen content were collected manually from four places and later homogenised and pooled. Collections were performed at four-hour intervals on days 21, 22 and 23 of each period. On day 21, the first sample was collected before the morning feeding (time 0) and at 4 and 8 hours afterwards. On day 22 the samples were collected at 1, 5 and 9 hours after the morning feeding. Finally, on day 23 the samples were collected at 2-, 6- and 10-hours after the sheep were fed in the morning. Thus, their rumen contents were collected before feeding and at 1-, 2-, 4-, 5-, 6-, 8-, 9-, and 10-hours post feeding. After removing the rumen digesta, the rumen content was filtered through four layers of cheesecloth. The solid part was returned to the rumen. The ruminal fluid was homogenised and the pH was measured. Then, two 20 mL aliquots were placed in plastic containers containing 1 mL of 6N HCl and duly identified. These samples were stored at -20 °C for later quantification of ammonia N (NH₃-N) according to Chaney and Marbac, (1962).

To calculate the total rumen content and density of the digesta, the rumen was emptied before and four hours after each feeding, and the contents were weighed. The density of the rumen contents was calculated as the ratio of mass to volume. The apparent DM passage rate (h) was calculated from the relationship between rumen content (Q) (kg DM) and feed intake (F) (kg DM/h) where $T(h) = Q/F$ (Cannas *et al.*, 2003)

Urine samples were obtained approximately four hours after feeding during spontaneous urination from the 21st through the 23rd day of each period. An aliquot of urine from each animal was filtered and packed in plastic pots and stored at -20 °C immediately after collection for further biochemical analyses.

In determining N balance, the ingested N and excreted N were measured by the micro Kjeldahl method. The absorbed N was calculated as the difference between ingested N and excreted N, and the retained N was calculated as the difference between the ingested N and excreted N.

Using PROC Mixed of SAS (SAS Institute, Inc., Cary, North Carolina, USA) the data were subjected to analysis of variance. Tukey's test at 5% probability was used to examine differences between the treatment means. The model was:

$$Y_{ijkl} = \mu + T_i + Q_j + P_k + A_{lj} + TQ_{ij} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = an observation,

μ = mean,

T_i = fixed effect of treatment i ,

Q_j = fixed effect of square j ,

P_k = random effect of period k ,

A_{lj} = random effect of animal l in square j ,

TQ_{ij} = interaction of treatment i and square j , and

ε_{ijkl} = random residual error.

Results and Discussion

The DM intake of sheep whose diets were supplemented with SBM, CSM and WCS were similar ($P > 0.05$) and about 22% higher ($P < 0.05$) compared with animals provided with U (Table 3).

Table 3 Intake and apparent digestibility of nutrients by sheep fed various nitrogen sources in spineless cactus-based diets

	Nitrogen sources				SE	<i>P</i> -value
	Soybean meal	Cottonseed meal	Whole cottonseed	Urea		
Intake, kg/day						
Dry matter	1.47 ^a	1.41 ^a	1.44 ^a	1.18 ^b	0.19	0.033
Organic matter	1.32 ^a	1.28 ^a	1.32 ^a	1.08 ^b	0.18	0.034
Crude protein	0.22 ^a	0.21 ^a	0.22 ^a	0.18 ^b	0.03	0.063
Neutral detergent fibre	0.65 ^a	0.61 ^a	0.68 ^a	0.47 ^b	0.09	0.001
Acid detergent fibre	0.29 ^b	0.29 ^b	0.34 ^a	0.20 ^c	0.04	<0.001
Ether extract	0.03 ^c	0.03 ^c	0.07 ^a	0.05 ^b	0.01	<0.001
Non-fibrous carbohydrates	0.40	0.40	0.37	0.44	0.06	>0.050
Apparent digestibility, kg/kg						
Dry matter	0.63 ^a	0.60 ^a	0.49 ^b	0.68 ^a	0.07	0.001
Organic matter	0.67 ^a	0.63 ^a	0.50 ^b	0.68 ^a	0.10	0.012
Crude protein	0.73 ^a	0.69 ^a	0.65 ^b	0.78 ^a	0.06	0.002
Neutral detergent fibre	0.63 ^a	0.54 ^b	0.48 ^b	0.62 ^a	0.07	0.002
Ether extract	0.60 ^b	0.77 ^a	0.82 ^a	0.78 ^a	0.43	0.019
Non-fibrous carbohydrates	0.73 ^{ab}	0.79 ^{ab}	0.66 ^b	0.86 ^a	0.12	0.042

^{a,b,c} With a row, means followed by a common superscript were not different with probability $P = 0.05$

The intakes of CP were similar for sheep supplemented with SBM, CSM, and WCS, but the intakes of ADF and EE were higher ($P < 0.05$) for WCS. Lower digestibility ($P < 0.05$) of DM and CP was also observed for WCS. The digestibility of DM, CP, neutral detergent insoluble fibre, and non-fibrous carbohydrates for the U diet was similar ($P > 0.05$) to the SBM diet.

The mass of rumen contents of sheep fed the SBM and CSM diets was similar ($P > 0.05$) and about 27% greater than that observed for animals fed with WCS and U diets. There was no difference ($P > 0.05$) among N sources for the density of rumen contents (Table 4).

Table 4 Characteristics of rumen fermentation of sheep fed various sources of nitrogen in spineless cactus-based diets

	Soybean meal	Cottonseed meal	Whole cottonseed	Urea	SE	<i>P</i> -value
Volume, L						
0 h	4.96 ^b	5.67 ^a	5.59 ^a	5.44 ^a	0.36	0.007
4 h	6.01 ^a	5.43 ^b	4.96 ^b	4.97 ^b	0.51	0.007
Mass, kg						
0 h	4.51 ^b	5.11 ^a	4.36 ^b	4.11 ^b	0.84	0.007
4 h	5.62 ^a	5.16 ^a	4.23 ^b	4.24 ^b	0.46	0.005
Density, kg/L						
0 h	0.90 ^a	0.90 ^a	0.77 ^b	0.75 ^b	0.08	0.001
4 h	0.93	0.95	0.86	0.85	0.08	>0.050
Total digesta						
Dry matter, g/kg digesta						
0 h	85.6	111.4	118.9	117.1	6.95	>0.050
4 h	114.3 ^{bc}	119.3 ^{ab}	122.2 ^a	110.0 ^c	5.01	0.001
Neutral detergent fibre, g/kg DM						
0 h	534.2	586.7	588.9	554.0	74.02	>0.050
4 h	549.8	564.9	543.1	535.6	41.18	>0.050
Crude protein, g/kg DM						
0 h	181.3 ^a	175.7 ^{ab}	171.8 ^{bc}	166.2 ^c	5.89	0.006
4 h	195.2 ^a	182.2 ^b	171.7 ^{bc}	166.8 ^c	10.52	<0.001
Rate of DM passage, %h	8.62	10.48	9.36	9.28	1.92	>0.050
Rate of DM disappearance, h	0.09	0.11	0.12	0.11	0.03	>0.050

^{a,b,c} Within a row, means followed by a common superscript were not different with probability $P = 0.05$
DM: dry matter

The DM of rumen content four hours after feeding was approximately 10 g/kg higher ($P < 0.05$) for sheep fed with CSM and WCS compared with animals fed with U. Crude protein was also higher ($P < 0.05$) in the rumen content of sheep fed with SBM and CSM compared with animals fed with U. No differences ($P > 0.05$) were found between N sources for the rate of pass or disappearance of DM.

The pH of rumen fluid from animals fed WCS and U differed ($P < 0.05$) from the sheep fed SBM and CSM (Figure 1) and the effect of feeding time was also significant ($P < 0.05$). All diets produced peak concentrations of rumen $\text{NH}_3\text{-N}$ between the first and second hours after feeding, followed by a decline (Figure 1). On average, the $\text{NH}_3\text{-N}$ in the ruminal fluid of sheep fed with WCS was similar ($P > 0.05$) to sheep fed with U and higher than the levels of sheep fed the SBM and CSM diets.

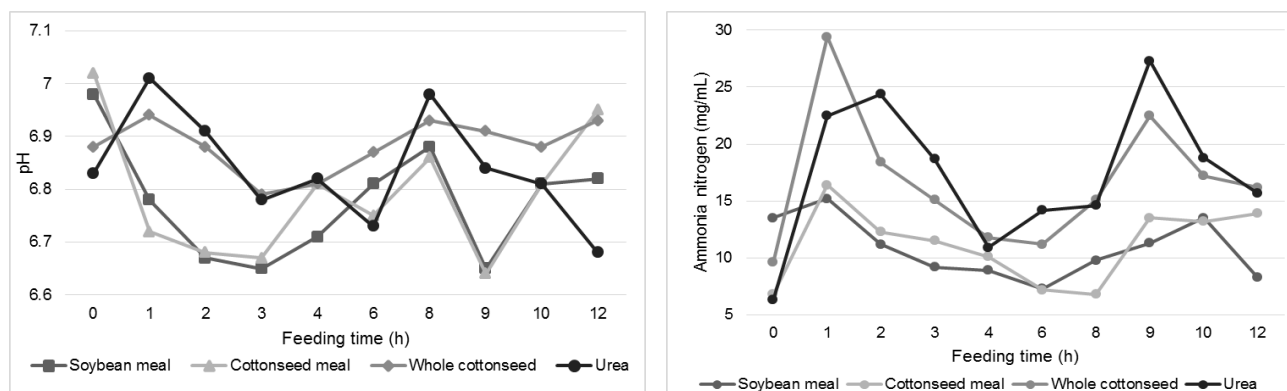


Figure 1 Rumen pH and ammonia nitrogen in sheep fed diets based on spineless cactus with various sources of nitrogen

Nitrogen intake by sheep fed the diet that contained WCS diets was higher than those that were fed the diet augmented with U ($P < 0.05$), with the SBM and CSM augmented diets producing intermediate responses (Table 5). The animals fed with U had lower ($P < 0.05$) faecal excretion of N and higher ($P < 0.05$) percentage of retained N than the other treatments. No effects ($P > 0.05$) of the N source on the N excretion in the urine, absorbed N or retained N (g/day) were observed.

Table 5 Nitrogen balance by sheep fed various nitrogen sources in diets based on spineless cactus

	Nitrogen sources				SE	<i>P</i> -value
	Soybean meal	Cottonseed meal	Whole cottonseed	Urea		
N intake, g/day	31.61 ^{ab}	29.60 ^{ab}	33.87 ^a	28.01 ^b	4.68	0.107
Excreted N, g/day						
Faeces	8.70 ^a	9.77 ^a	11.02 ^a	5.88 ^b	2.09	0.001
Urine	6.54	5.77	5.40	5.75	1.25	>0.050
Absorbed N, g/day	22.90	19.82	22.85	22.12	3.75	>0.050
Retained N, g/day	16.36	14.04	17.44	16.37	3.46	>0.050
% consumed	51.75 ^b	47.43 ^b	51.49 ^b	58.44 ^a	7.19	0.020
% absorbed	71.40	70.80	76.32	74.01	7.59	>0.050

^{a,b}With a row, means followed by a common superscript were not different with probability $P = 0.05$

Dry matter intake was similar for the animals for that were fed the diets with SBM, CSM, and WCS. However, animals fed with U had a lower DM intake, possibly owing to the lower palatability of this diet. Urea reduced the DM intake of animals when added in large quantities to ruminant rations (Kozloski *et al.*, 2007; Rebelo *et al.*, 2019). In the present study the animals consumed about 0.5 g of urea per kg of bodyweight, a high amount that has been described as intoxicating for hungry animals (Antonelli *et al.*, 2009). The hypothesis of reduced diet palatability was reinforced by the observations that neither the digestibility DM nor the rate of its disappearance from the rumen was reduced in the animals fed with urea. Miller-Cushon *et al.* (2014) also found that the type of N source affected palatability and feed intake by calves.

The differences in source of protein were not sufficient to cause variations in protein intake. However, CP digestibility was lower in WCS. Whole cottonseed has bark with a high degree of lignification (10% lignin) (Bertrand *et al.*, 2005), which must be broken so that the microorganisms have access to the c and its nutrients. Since a large amount of whole seed was seen in the faeces, it was proposed that reduced protein digestibility was because of undigested seed. Faecal N of animals fed with WCS was about 47% higher than those fed with U, supporting the hypothesis of reduced digestibility. Silva *et al.* (2016) also observed a reduction in the DM, CP, and NDF digestibility when SBM was replaced with cottonseed cake in diets for sheep.

The reduced digestibility of NDF with CSM and WCS diets may be because of the presence of cotton husk, which provides more lignin in the NDF of these feedstuffs (Goes *et al.*, 2011). In addition, the low NDF digestibility in the WCS diet might be owing to the increased intake of EE. Costa *et al.* (2012) also observed reduced NDF and CP intake and DM digestibility in sheep fed with WCS in diets based on spineless cactus.

The presence of urea in WCS and U diets might have contributed to the higher rumen pH compared with SBM and CSM, especially immediately after feeding. The presence of urea also contributed to the higher NH₃-N levels in the rumen fluid observed in sheep fed with the WCS and U diets. The maximum NH₃-N concentration in the rumen fluid occurred two hours after feeding, regardless of the N source. Similarly, Pessoa *et al.* (2013) found no differences in pH and NH₃-N in the rumen of sheep fed various protein sources.

The volume of the digesta at four hours after feeding was about 1 L higher for SBM than the animals fed U, but the DM contents of the digesta were similar for the two diets at this time. The lower intake of the animals fed the U diet probably contributed to the similarity in digestibility of DM between the U and SBM diets, even though the volumes of rumen contents differed. When feeding diets based on spineless cactus, Souza *et al.* (2009) did not observe any effect of various fibre sources on the rate of disappearance of DM from the rumen of goats.

The CP content of rumen contents was affected by the N source, possibly owing to the differences in fractionation and degradation rates of protein from SBM, CSM, and U. Soybean meal has a high rate of degradation in the rumen, which contributes to greater microbial growth (Marcondes *et al.*, 2009). However, CSM has a slower degradation rate than SBM, possibly owing to the presence of its lignified husk, which is potentially why lower digestion of crude protein was observed when feeding CSM as opposed to SBM (Queiroz *et al.*, 2010).

The rapid conversion of urea to ammonia and its immediate absorption from the rumen can explain why the CP level of the digesta of urea-fed sheep was lower than those fed the SBM and CSM diets (Ahvenjärvi & Huhtanen, 2018; Spanghero *et al.*, 2018). The rapid absorption of ammonia when feeding the U diet also helped to explain the low recovery of N in faeces and higher N retention. This result corroborated the recent meta-analysis of Schuba *et al.* (2017), who found that the intake of N was strongly related to faecal N. The association of the spineless cactus with urea could also provide a better synchronisation of rumen degradation and greater efficiency of N use, as was observed by Santos *et al.* (2020).

Conclusion

Soybean meal and cottonseed meal are similar in their effects on animals and can be used for sheep in diets based on spineless cactus. Whole cottonseed and urea are interesting sources of N, but should be used with caution owing to their depressing effects on the digestibility and nutrient intake of sheep.

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Authors' Contributions

ACAP (Orcid 0000-0001-9987-2631), DBC (Orcid 0000-0002-7137-534X) and KCS (Orcid 0000-0002-3476-6085) participated in designing the study, laboratory analysis, and manuscript writing. RASP (Orcid 0000-0001-5361-0214), AMVB (Orcid 0000-0001-6133-2795) and ASCV (Orcid 0000-0002-7673-0654) assisted in drafting and revising the manuscript for important intellectual content. FFRC (Orcid 0000-0001-9211-0263) and DMLJ (Orcid 0000-0002-1154-8579) conducted the data analysis and interpretation.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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