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Effects of dietary inclusion of Tongmai granule residue on growth performance, nutrient digestibility, blood biochemical parameters and rumen fermentation in sheep

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ABSTRACT. The aim of the present study was to investigate the effects of dietary inclusion of Tongmai granule residue (TGR) on growth performance, nutrient digestibility, blood biochemical parameters and rumen fermentation in sheep. Twenty-four healthy Doper × Small Tail Han hybrid female lambs (6–7 months of age; 29.64 ± 1.80 kg initial body weight) were randomly allocated into 3 groups and fed diets to which 0, 10 and 15% TGR were added for 75 days. It was shown that TGR dietary inclusion at the level of 15% decreased growth performance, neutral detergent fibre apparent digestibility, rumen contents of acetic, propionic, butyric acids and total volatile fatty acids ($P < 0.05$), but increased crude fat and organic matter (OM) apparent digestibilities, serum high-density lipoprotein cholesterol, immunoglobulin (Ig)A, IgG, IgM, rumen pH, NH_3-N concentration and acetic:propionic acid ratio in lambs ($P < 0.05$). In the 10% TGR group, OM apparent digestibility and IgA also increased ($P < 0.05$), however, no negative effects on average daily weight gain, rumen pH, NH_3-N, volatile fatty acids concentration and blood biochemical parameters were observed. So, it is suggested that TGR could be used as a feed source in sheep production, and 10% TGR inclusion is the most suitable dose.

Introduction

Chinese herbal medicine has been used for more that 2000 years to prevent and treat many diseases. The advantages of herbs are also used as health and production promoters in the animal husbandry. Herbs provide such nutrients as peptides, proteins, essential amino acids, starch, oligosaccharides, vitamins, fatty acids and organic trace minerals, and as well, possess many bioactive ingredients with antibacterial, immune enhancement and stress reduction properties (Guo et al., 2004). Also, previous studies on Chinese herbs as feed additives have shown that these herbs can modulate the immune response, nutritional metabolism and intestinal health of food-producing animals (Gong et al., 2014).

In recent years, the Chinese herbal medicine industry has been rapidly developed due to the continuous increase in the application of Chinese herbal medicine resources. This extensive use has resulted in an increased yield in Chinese herbal medicine residue. Moreover, due to poor extraction efficiency, the herbal residue still contains more than 30–50% medicinally active ingredients (Meng et al., 2017). Tongmai formula is a popular Chinese herbal medicine preparation composed of three traditional

Chinese herbs: *Chuanxiong rhizoma* (rhizomes of *Ligusticum chuanxiong*), *Puerariae lobatae* radix (root of *Pueraria lobata*) and *Salviae miltiorrhizae* radix (root of *Salvia miltiorrhiza*) in a ratio of 1:1:1. It is widely used in the treatment of cerebrovascular and cardiovascular diseases, such as high blood lipids, myocardial infarction and atherosclerosis (Wu et al., 2017). It was indicated that the *Puerariae lobatae* radix and *Chuanxiong rhizoma* residue contain 69.9% puerarin (Huang, 2009) and more than 30% tetramethylpyrazine (Xu, 2005), respectively. *Salviae miltiorrhizae* radix residue contains 3.62% tanshinone IIA, 2.56% cryptotanshinone, 2.75% tanshinone and 1.02% methylenetanshinone (Shi et al., 2010). Li (2015) found that diets supplemented with 1% *Salviae miltiorrhizae* radix had no significant effects on growth performance and feed efficiency but improved carcass performance, meat quality and caecal microflora, regulated the nutritional composition in muscle and increased antioxidant status and activities of digestive enzymes of growing pigs. Feeding *Puerariae lobatae* radix to cow would increase milk yields and milk protein, calcium, magnesium, iron, zinc, copper and total flavonoids concentrations (Zhang, 2011). The functions of tetramethylpyrazine, such as anti-inflammation, antioxidant, antiplatelet and antiapoptosis, as well as boosting immunity in humans and animals were also confirmed (Gao et al., 2015; Zhao et al., 2016).

So far the positive effects of *Chuanxiong rhizoma*, *Puerariae lobatae* radix and *Salviae miltiorrhizae* radix on animals had been confirmed; however, the research on Tongmai formula is mainly focused on its chemical composition. No research has been conducted on the application of Tongmai granule residue (TGR) in animal production, the comprehensive effects of *Chuanxiong rhizoma*, *Puerariae lobatae* radix and *Salviae miltiorrhizae* radix in TGR still need to be explored. Therefore, the main objective of the present study was to determine the effects of TGR on the growth performance, apparent nutrient digestibility, blood biochemical parameters and rumen fermentation in Doper × Small Tail Han hybrid sheep, and to explore the feasibility of using TGR as a ruminant feed ingredient.

Material and methods

All experimental protocols involving animals were approved by the Institutional Committee for Animal use and Ethics of the College of Animal Nutrition and Feed Science of Jilin Agricultural University (Changchun, China) and also in agreement with the provincial rules and regulations.

Tongmai granule residue

Fresh TGR which mainly consists of *Salvia miltiorrhiza*, *Ligusticum chuanxiong* and *Pueraria lobata* were obtained from Xiuzheng Pharmaceutical Co. Ltd. (Tonghua, China). The residue was air-dried and then pulverized to pass through a 2-mm screen. The nutrient contents of TGR are shown in Table 1.

Table 1. Nutrient contents of Tongmai granule residue, g/kg dry matter

Indices	Content
Organic matter	823.0
Crude protein	111.3
Ether extract	19.4
Neutral detergent fibre	542.3
Acid detergent fibre	445.4
Acid detergent lignin	149.4
Crude fibre	336.2

Animals and feeding management

In total, twenty-four 6–7 month old crossed Doper × Small Tail Han hybrid ewes with an average weight of 29.64 ± 1.80 kg (weaned at 3 months old) were randomly allocated to 3 treatment groups (8 sheep per treatment) in a completely randomized block design based on body weight. Each group was fed a diet containing different levels of TGR: 0, 10 and 15%. Animals were arranged in a covered area and allotted into individual pens of suspended slatted floors. The diets were formulated according to the National Research Council (NRC, 2011) recommendations for an average daily weight gain of 200 g/day (Table 2). The experiment lasted 75 days with an adaptation period of 15 days. During this period, the animals were weighed, tagged and treated for ecto- and endoparasites. Diets were offered twice a day (8:00 and 16:00) with free access to clean drinking water. Feed and water troughs were cleaned daily and pens were kept ventilated and hygienic at all times.

Sampling and analysis

The animals were weighed before the morning feeding on the first and last day of the feeding period, and the amount of feed supplied and refused was recorded per animal. The average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) were calculated.

On day 65 of the feeding period, 6 sheep in each group were transferred to single metabolic cages

Table 2. Ingredients and chemical composition of the experimental diets

Indices	Diet		
	Control (0% TGR)	10% TGR	15% TGR
Ingredients, g/kg DM			
guinea grass	300.0	300.0	300.0
maize straw	100.0	100.0	100.0
TGR	0.0	100.0	150.0
molasses	18.0	18.0	18.0
maize	255.0	210.0	204.0
distillers dried grains with solubles	109.5	95.2	93.5
maize gluten meal	175.0	133.8	90.8
premix[1]	4.0	4.0	4.0
urea	3.5	5.0	6.7
sodium chloride	8.0	8.0	8.0
calcium carbonate	15.0	15.0	15.0
dicalcium phosphate	4.0	4.0	4.0
sodium bicarbonate	8.0	7.0	6.0
Nutrient content[2], g/kg DM			
DM, g/kg fresh weight	886.0	888.0	889.0
organic matter	823.0	824.0	824.0
crude protein	124.4	123.9	123.8
ether extract	35.5	35.4	35.3
ME , MJ/kg DM	9.9	9.7	9.6
neutral detergent fibre	418.0	457.0	464.0
acid detergent fibre	256.0	272.0	288.0
calcium	8.2	8.7	9.0
phosphorus	5.0	4.6	4.2

TGR – Tongmai granule residue, DM – dry matter, ME – metabolizable energy; [1] provided per kg of diet: IU: vit. A 15 000, vit. D 2 000, vit. E 55; mg: Fe 50, Co 0.2, Cu 12.0, Se 0.5, Mn 50, I 0.55, Zn 25; [2] measured values except ME

for the metabolic test. Leftovers and total faeces samples were collected before the morning feeding from each animal from day 68 to 74 of the feeding period. Samples of approximately 10% of the total faeces were taken and 10% H_2SO_4 were added. At the end of the metabolic test period, the samples were composted, dried at 65 °C for 72 h, weighed, ground to pass through a 1-mm screen and then analysed for crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) and organic matter (OM).

Blood samples were collected from the jugular vein of each sheep into 5-ml heparinized collection tubes before the morning feeding on day 60 of the experiment. The blood samples were centrifuged at 3500 *g* for 10 min at 4 °C. The serum was obtained and stored at −80 °C until further analysis. Serum blood urea nitrogen (BUN), glucose (GLU), total cholesterol (TC), triglycerides (TG), total protein (TP), high-density lipoprotein cholesterol (HDL-C), albumin (ALB), low-density lipoprotein cholesterol (LDL-C) and globulin (GLB) concentrations were determined on a fully automatic biochemical analyzer using a standard commercial kit (Jilin Jinyu Medical Laboratory Co., Ltd., Changchun, China). The concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) in serum were analyzed using commercial ELISA kits supplied by the Nanjing Jian Cheng Bioengineering Institute (Nanjing, China).

Ruminal fluid samples were obtained from the rumen immediately after the sheep were slaughtered at the end of the experiment (6 sheep in each group). Ruminal pH was measured using a portable type pH meter (S20K, Mettler Toledo, Columbus, OH, USA) during rumen content collection. The samples were then filtered through four layers of cheesecloth and the extracts were collected into 50-ml centrifuge tubes and then centrifuged at 10 000 *g* for 15 min at 4 °C. The supernatants were collected and subsequently stored at –80 °C until analyses for ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations. The NH_3-N concentration was determined by spectrophotometer colourimetry, and the VFA concentration was measured by gas chromatography.

Statistical analysis

Data were analyzed by analysis of variance, using the General Linear Model procedure of SPSS 23.0 (SPSS Inc., Chicago, IL, USA). The means of each trait were compared by Tukey multiple comparisons and presented with the standard error of the mean. Differences were considered statistically significant if $P \leq 0.05$.

Results

Growth performance

The effect of dietary inclusion of TGR on growth performance in Doper × Small Tail Han sheep is presented in Table 3. The growth performance of sheep was not improved by TGR dietary inclusion.

Table 3. Effects of dietary supplementation of Tongmai granule residue (TGR) on sheep growth performance

Indices	Diet			SEM	*P*-value
	Control (0% TGR)	10% TGR	15% TGR		
Initial weight, kg	33.2	31.5	31.3	0.72	0.52
Final weight, kg	41.4	40.1	38.6	0.72	0.31
ADG, g/day	137[a]	144[a]	121[b]	3.95	0.02
DMI, kg/day	1.51	1.46	1.50	0.03	0.71
FCR	11.05[b]	10.2[b]	12.5[a]	0.36	0.01

ADG – average daily gain, DMI – dry matter intake, FCR – feed conversion ratio, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

When TGR was added at the level of 15%, ADG was significantly decreased ($P < 0.05$), and FCR was significantly increased ($P < 0.05$). There was no significant difference in DMI among sheep groups ($P > 0.05$).

Apparent nutrient digestibility

As shown in Table 4, the dietary inclusion of TGR had no significant effect on the apparent digestibility of CP and ADF ($P > 0.05$). However, 10% TGR significantly increased the apparent digestibility of OM ($P < 0.05$), while 15% TGR significantly increased the apparent digestibility of EE and OM ($P < 0.05$), and significantly decreased that of NDF ($P < 0.05$).

Table 4. Effects of dietary supplementation of Tongmai granule residue (TGR) on apparent nutrient digestibility in sheep, g/kg

Indices	Diet			SEM	*P*-value
	Control (0% TGR)	10% TGR	15% TGR		
CP	701	684	672	6.16	0.15
EE	556^{b}	626^{ab}	694^{a}	22.9	0.03
OM	504^{b}	638^{a}	581^{a}	19.0	<0.01
ADF	426	417	381	18.8	0.63
NDF	668^{a}	617^{ab}	594^{b}	13.7	0.04

CP – crude protein, EE – ether extract, OM – organic matter, ADF – acid detergent fibre, NDF – neutral detergent fibre, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Blood biochemical parameters

The effect of TGR on the blood biochemical parameters in sheep is summarized in Table 5. TGR dietary inclusion had no significant effect on serum GLU, BUN, TG, TC, LDL-C, TP, ALB and GLB concentrations in all dietary groups ($P > 0.05$). Dietary inclusion of 10 and 15% TGR significantly increased serum IgA concentration while only 15% TGR dietary inclusion increased the concentrations of HDL-C, IgG and IgM in serum ($P < 0.05$).

Rumen fermentation parameters

As shown in Table 6, TGR at 10% inclusion had no significant effect on rumen pH, NH_3-N, total VFA, acetic, propionic and butyric acids concentrations as well as the acetic:propionic acid ratio in the rumen ($P > 0.05$). However, TGR at 15% inclusion significantly decreased the contents of acetic, propionic, butyric acids and total VFA ($P < 0.05$) and significantly increased pH, NH_3-N concentration, and the acetic:propionic acid ratio ($P < 0.05$) in the rumen.

Table 5. Effects of dietary supplementation of Tongmai granule residue (TGR) on blood biochemical parameters in sheep

Indices	Diet			SEM	*P*-value
	Control (0% TGR)	10% TGR	15% TGR		
GLU, mmol/l	4.11	3.92	4.06	0.08	0.59
BUN, mmol/l	5.48	5.42	5.35	0.14	0.95
TG, mmol/l	0.40	0.42	0.43	0.01	0.22
TC, mmol/l	1.90	1.89	2.11	0.07	0.34
HDL-C, mmol/l	1.20^{b}	1.21^{b}	1.41^{a}	0.04	0.04
LDL-C, mmol/l	0.59	0.62	0.66	0.02	0.45
TP, g/l	66.4	66.4	66.4	0.75	1.00
ALB, g/l	36.8	35.6	34.50	0.56	0.27
GLB, g/l	29.60	30.8	32.00	1.00	0.65
IgA, ug/ml	53.8^{b}	62.01^{a}	63.9^{a}	1.78	0.03
IgG, ug/ml	131^{b}	130^{b}	190^{a}	8.07	<0.01
IgM, ug/ml	360^{b}	371^{ab}	391^{a}	5.31	0.04

GLU – glucose, BUN – blood urea nitrogen, TG – triglyceride, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, TP – total protein, ALB – albumin, GLB – globulin, IgA – immunoglobulin A, IgG – immunoglobulin G, IgM – immunoglobulin M, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Effects of dietary supplementation of Tongmai granule residue (TGR) on rumen fermentation parameters in sheep

Indices	Diet			SEM	*P*-value
	Control (0% TGR)	10% TGR	15% TGR		
Rumen pH	5.83^{b}	5.89^{b}	6.31^{a}	0.09	0.04
NH_3-N, mg/dl	3.51^{b}	3.17^{b}	6.71^{a}	0.32	<0.01
Total VFA, mmol/l	95.6^{a}	98.7^{a}	62.3^{b}	5.34	<0.01
Acetic acid, mmol/l	59.3^{a}	63.2^{a}	41.4^{b}	3.60	<0.01
Propionic acid, mmol/l	22.6^{a}	21.6^{a}	10.7^{b}	2.04	<0.01
Butyric acid, mmol/l	8.91^{a}	9.92^{a}	7.08^{b}	0.45	<0.01
Acetic:Propionic acid	2.63^{b}	2.97^{b}	3.89^{a}	0.21	<0.01

VFA – volatile fatty acids, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

Salviae miltiorrhizae radix supplementation did not affect growth performance and utilization of nutrients, but improved carcass performance, meat quality and increased antioxidant status in growing pigs (Li, 2015). Meanwhile, in some studies an increased growth performance in sheep (Guo et al., 2010) and cattle (Xia et al., 2009) was observed. Puerarin has been shown to inhibit the appetite of rats, reduce body weight and improve lipid metabolism. Reports on the effects of *Ligusticum chuanxiong* on animal growth performance were not found. The present experiment indicated that dietary inclusion of 10% TGR did not have any negative effect on the growth performance of sheep. However, the addition of 15% TGR decreased the DMI, ADG and increased the FCR in sheep. This may have been related to the effect of puerarin, an active ingredient in Tongmai granule, on weight loss and regulation of fat metabolism (Wang et al., 2017). However, its related mechanism needs further study.

Digestibility is a major indicator of the absorption and utilization of feed nutrients in animals and is influenced by factors such as feed quality and animal feeding (Lin et al., 2006). TGR contains more than 11% CP, and also 54.23% NDF, 44.54% ADF and 14.94% ADL (Table 1). Therefore, using the proportion of 10 and 15% TGR in the diet will inevitably affect the composition of nutrients, such as protein and fibre, and then affect nutrients digestibility. In the present study, the digestibilities of OM and EE increased, but NDF decreased with the TGR addition. Shang et al. (2018) found that dietary addition of puerarin can promote fat metabolism and improve apparent digestibility of OM in beef cattle under high-temperature conditions, and the results of the present study confirm this finding. The decrease in NDF digestibility may have resulted from the changes in the diet fibre source since 10 and 15% TGR replaced part of the maize dried distillers grains with solubles (DDGS) and maize gluten meal in the control diet. Another possible reason for the decrease in dietary NDF digestibility may be due to the increase in dietary NDF and ADF levels with the TGR addition. At present, there is little research on the effect of TGR or TGR's components on the apparent digestibility in ruminants. The mechanism of TGR effects on nutrient digestibility in sheep still needs to be explored.

Blood biochemical parameters usually reflect the health status of an animal. These parameters are vital indicators of the physiological and nutritional status of animals (Alagawany and EI-Hack, 2015). *Salviae miltiorrhizae* radix as one of the main components of TGR was reported to reduce the patient serum levels of TG, TC, LDL-C and UA, but to increase the levels of serum HDL-C after three months of administration (Liu et al., 2016). Puerarin, a major isoflavone compound present in *Pueraria lobata*, has a variety of biological actions useful in treating cardiovascular and gynaecological diseases, osteoporosis, diabetic nephropathy and problems with cognitive capability. Many reports have demonstrated that puerarin possesses a lot of activities including antioxidative activity, anti-inflammation and antiapoptosis (Guo et al., 2013; Li et al., 2013). It was shown that puerarin markedly decreased the TC in serum and liver (Chung et al., 2008; Noh et al., 2011). Positive influence of *Ligusticum chuanxiong* on cardiovascular and cerebrovascular conditions, antioxidation, neuroprotection, antifibrosis, antinociception, anti-inflammation and antineoplastic activity was presented. *Ligusticum chuanxiong* can lower the levels of serum TG, TC but raise the levels of HDL-C. In the current study, dietary inclusion of TGR had no significant effect on serum TC and TG concentrations but increased HDL-C level. It was shown that the residual active ingredients in TGR still played a role in regulating lipid metabolism and also increasing the serum HDL-C level of sheep. No significant change in sheep serum TG and TC levels may be related to the residual amount and composition of active ingredients in TGR, feeding time and feeding amount of TGR. Blood GLU, TP, ALB, GLB and BUN levels are a direct response of animals' carbohydrate and protein digestion and metabolism. Dietary inclusion of TGR did not affect sheep serum TP, ALB, GLB and BUN levels in the present study. At the same time, the CP digestibility did not significantly differ among the diet treatments. Although *Pueraria lobata* is related to a decrease in blood GLU (Xie, 2018), no significant decrease in blood GLU was observed in the present experiment.

Immunoglobulin molecules (IgA, IgG and IgM) which are associated with humoral immunity are produced by the differentiation and proliferation of B lymphocytes, which reflects the status of the body immune function (Li et al., 2016; 2018). In this study, dietary inclusion of TGR increased the content of serum IgM, IgA and IgG in sheep. This indicates that TGR can increase the number of lymphocytes and improve the rate of lymphocyte transformation. Furthermore, the increase in immunoglobulin content reflects the enhancement of humoral immune function in sheep. These results were consistent with the studies on *Salvia miltiorrhiza*. He (2008) indicated that *Salvia miltiorrhiza* can enhance the immune function of mice by promoting the proliferation of splenic lymphocytes.

At present, no reports have been found on the effect of TGR or its ingredients on rumen fermentation. In this study, it was found that 10% TGR inclusion into sheep diet had no significant effect on rumen total VFA, acetate, propionate and butyrate concentrates. However, when 15% TGR was added, not only the concentrations of acetate, propionate and butyrate decreased but also the proportions of acetate and propionate increased.

Volatile fatty acids are the end product of carbohydrates in the rumen and provide 70 to 80% of the digestible energy in ruminants. In general, roughages (structural carbohydrates) produce a high proportion of acetic acid in the rumen, while concentrates (non-structural carbohydrates) produce a high proportion of propionic acid in the rumen (Hao et al., 2011). In the present study, a similar phenomenon was found as 15% TGR inclusion increased rumen acetate proportion and decreased propionate proportion, with a corresponding increase in the NDF and ADF contents. As propionic acid is the main precursor of gluconeogenesis, the GLU produced by propionic acid can meet about 30–50% energy requirement of sheep. The decrease in propionate proportion in this study probably resulted in a decrease in energy utilization. In addition, if butyric acid is the only available energy source, it can be completely utilized by the colonic mucosa to produce ketone bodies and CO_2. When acetic, propionic and butyric acids are absorbed by colon mucosal epithelial cells, they can be consumed by intestinal mucosal epithelial cells as energy. Therefore, the ratio of acetic, propionic and butyric acids in the rumen VFA has a direct impact on the energy conversion efficiency (Hao et al., 2011). The changes in VFA proportion would be an interesting question that needs further studies especially if the changes could be attributed to TGR addition into sheep diet in the future.

The decreased rumen VFA concentration led to an increase in rumen pH and NH_3-N concentration when 15% of TGR was added to the diet. NH_3-N is the main source of nitrogen (N) in the process of microbial protein synthesis in the rumen and plays a vital role in the efficient microbial fermentation of feed. The level of ruminal NH_3-N represents a balance between microbial protein synthesis and rumen protein degradation (Wang et al., 2016). Previous studies indicated that the optimal concentration of NH_3-N in the rumen is 0.35–29 mg/dl (Owens and Bergen, 1983). In the current study, the rumen NH_3-N concentration in each group was within the normal range. Volatile fatty acids are the main products of carbohydrate fermentation by various microorganisms in the rumen. They play the role of maintaining the rumen environment and providing energy for the animals' body. In the present study, dietary inclusion of 10% TGR did not affect the concentration of total VFA but 15% TGR significantly decreased rumen total VFA indicated that excessive TGR addition will impair rumen carbohydrate fermentation. This was confirmed by the decrease in NDF digestibility in this study. The increase in pH may be due to the decrease in rumen total VFA. This indicates that the dietary addition of TGR has the potential to regulate the efficiency of nutrient digestion and the rumen fermentation pattern.

Conclusions

It was demonstrated that dietary inclusion of 10% Tongmai granule residue (TGR) did not have any negative effects on the growth performance, digestibility, blood biochemical parameters and rumen fermentation, but played a protective role in immune function in Doper × Small Tail Han hybrid female lambs. However, when 15% TGR was added, although high-density lipoprotein cholesterol and serum antibodies levels were increased, it had adverse effects on performance, nutrient digestion and rumen fermentation of sheep. Therefore, under the conditions of the current experiment, it can be concluded that the 10% TGR dietary inclusion is appropriate. Further studies should be conducted to validate the feeding periods, inclusion amounts and possible interaction effects of TGR addition to sheep diets.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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