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A Study on Relative Effect of CaCl2 on Physico-chemical Parameters of Breast and Thigh Muscles of Spent Hen Using Multivariate Analysis

C. O. Vinayananda[1*], R. Narendra Babu[2], V. Appa Rao[3], A. Kalaikannan[4], K. Vijayarani, S. Ramesh[6] and Serma Saravana Pandian[7]

[1]Ph. D Scholar, Department of Livestock Products Technology (Meat Science), Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[2]Professor and Head, Department of Livestock Products Technology (Meat Science), Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[3]Dean, College of Food and Dairy Technology, Koduvalli-Alamathi, TANUVAS, Chennai, Tamil Nadu, INDIA
[4]Assistant Professor, Veterinary University Training & Research Centre, TANUVAS, Thanjavur, Tamil Nadu, INDIA
[5]Professor and Head, Bioinformatic Centre & ARIS Cell, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[6]Professor and Head, Centralized Instrumentation Laboratory, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[7]Assistant Professor, Department of Animal Husbandry Economics, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA

*Corresponding Author: vinvet22@gmail.com

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Abstract

A study was conducted to assess the relative effect of CaCl2 on the breast and thigh muscles of spent White Leghorn layers. A total of 96 muscle samples obtained from 24 spent hens were used to study the effect of CaCl2 injection on physico-chemical properties of breast and thigh muscles. Physico-chemical parameters like pH, water holding capacity, protein extractability, shear force value and myofibrillar fragmentation indices of breast and thigh muscles significantly (P≤0.01) varied with injection of $CaCl_2$ with 24 hr of ageing. Significant (P≤0.01) difference was observed for L and a* values between control breast or thigh and corresponding treated muscles, no significant (P≥0.05) difference was observed for b* values between control and treated muscles. However, the color differences (ΔE) values among all groups were over the just-noticeable difference threshold. Discriminant function analysis and Mahalanobis distance (D2) analysis of physico-chemical parameters demonstrated that the effect of CaCl2 is comparatively greater in thigh muscle than breast muscle with an ageing period of 24 hr.*

Keywords: CaCl2, Color, Discriminant function analysis, Physico-chemical properties, Spent hen

Introduction

In last few decades, poultry and their products have become major components in the supply of animal foods for human consumption and their role is rapidly increasing, especially in developing countries. Although spent layer meat is economically important, it is considered as a by-product of the egg industry (Kondaiah and Panda, 1992). Unlike broiler, spent layer meat has limited scope because of its toughness and it has been a problem in the poultry industry with respect to marketing of such tough meat. Spent meat possesses several health promoting benefits due to its rich omega-3 fatty acids with low cholesterol and good source of protein especially the myofibrillar protein content (Ajuyah *et al*., 1992; Lee, 2003). Hence, it is necessary to improve the quality of tough meat for efficient utilization of the nutritive and health benefits while improving the financial returns to the farmers.

Among the sensory attributes of the meat, tenderness is considered as the most important quality affecting consumer satisfaction and reflects a positive perception of acceptability of meat (Gao *et al.*, 2016). It is well known that the process of meat tenderization is essentially an intrinsic enzymatic activity, dependent on pH, temperature, ionic strength etc. Enzymes tenderize the meat by weakening the intra- and interfibrillar bonds of myofiber and myofiber-associated proteins. The synergistic action of 3 proteolytic systems– cathepsins, the proteasome and calcium-dependent proteases has been proposed to be involved in tenderization by several authors (Thomas *et al*., 2004; Ouali *et al*., 2006). Many studies have reported that the calcium-dependent system or calpain system is the principal contributor in post-mortem proteolysis/ tenderization. Thus, tenderization processes can be accelerated by using calcium chloride ($CaCl_2$) (Wan *et al*., 2018). Chang and Chou (2010) reported that activity of calpains (μ and m) vary among the different muscles (breast and leg) of Taiwan black-feathered country chickens.

The meat quality is influenced by various pre- and post-slaughter factors (Mir *et al*., 2017) and intrinsic physical, biochemical and nutritional properties (Naveena *et al*., 2011; Suriani *et al*., 2014), that vary among different muscles within the same carcass. The biophysical, histological and biochemical characteristics of muscle fibres play a key role affecting the meat quality (Teimouri and Tumova, 2009). The skeletal muscle is composed of different types of muscle fibers, which vary according to their molecular, metabolic (glycolytic or oxidative), structural and contractile properties (Choi and Kim, 2009). In chicken, the breast muscle (*M. Pectoralis*) is composed of only type IIB muscle fibres (Iwamoto *et al*., 2003; Roy *et al*., 2006) and the thigh (*M. Biceps femoris*) is composed of Type I, IIA and IIB (Papinaho *et al*., 1996) fibers. With this view, the present work has been designed to study the relative effect of calcium chloride on physico-chemical parameters of the breast and thigh muscles of spent hens with the application of a multivariate statistical tool.

Materials and Methods

Sampling and Experimental Design

A total of 96 muscle samples (comprising 48 breast and thigh muscles each) obtained from 24 numbers of spent hens were used in the current study. White Leghorn birds reared under the same management conditions were procured at the age of approximately 72 weeks and weighing around 1.2-1.3 kg from the local market of Chennai, Tamil Nadu. Birds were withdrawn from feed for 12 hr and slaughtered as per standard protocol followed by the Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Chennai, Tamil Nadu, India. After dressing, the carcasses breast and thigh muscles were removed without affecting the integrity of muscle and divided into four groups i.e. Breast control (BC), Breast treatment (BT), Thigh control (TC) and Thigh treatment (TT). Muscles of the treatment groups were injected with 300mM calcium chloride solution at 10% (w/w) and the control groups were injected with double distilled water using a hand held single needle syringe. After injection, the muscles were kept at room temperature for 15 min and packed in sandwich bags and subjected to aging at 4±1 °C for 24 hr followed by analysis of physico-chemical and sensory properties.

Physico-chemical Parameters

Physico-chemical parameters of all groups of muscles were evaluated after 24 hr of treatment. The pH of the chicken meat samples was measured using a pre calibrated digital pH meter (Cypberscan 510, Eutech Inst., Singapore) according to Troutt *et al.* (1992). Water-holding capacity (WHC) was measured by the filter paper press method as per Grau & Hamm (1953). Area were measured using Placom KP–90N planimeter (Koizumi Sokki Mfg. Co. Ltd., Japan) and WHC was calculated as the ratio of meat area to fluid area on the filter-paper as per George *et al*. (1979).

Total protein extractability was determined according to procedure of Joo *et al*. (1999) and results were expressed as mg/g. Myofibrillar fragmentation index (MFI) values were determined by the procedure of Davis *et al*. (1980) and results were reported as the weight of the residue in grams times one hundred. Cooking loss was calculated as the difference in sample weight before and after cooking, and expressed as a percentage of the initial sample weight (Jama *et al*., 2008). The shear force values were assessed following the standardized protocol of wheeler *et al.* (1997). Where, both pectoral and thigh samples were trimmed to uniform size (30mm x 10mm x 8mm) with length parallel to the muscle fibers and sheared perpendicular to the muscle fiber using Warner Bratzler Meat Shear (G.R. Electric Manufacturing Company, Manhattan, USA) the average of the five readings were recorded and expressed as kgf.

Color and Sensory Evaluation

Meat color was measured with Spectro-colorimeter (Hunter color lab Mini scan XE plus, Model No. 45/O-L, Reston, Virginia, USA), calibrated prior to each session for the CIE color space system (CIE, Commission Internationale de l'Eclairage, 1976) using a white tile (L*: 94; a*: 1.10; b*:0.6) with geometry of diffuse/80 (sphere - 8mm view) and an illuminant of D65/10 deg. Measurements were performed in triplicate, taking the mean value as the assay result (Hunt *et al*., 1991). The numerical total color difference (ΔE) among control and treated samples of breast or thigh meat was calculated using the formula given by Mancini *et al*. (2015)-

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where "Δ" means the difference in color between the groups. A variation in color (ΔE) equal to 2.3 units corresponds to a just-noticeable difference (JND) for the human eye; higher variation is considered discernible (Sharma, 2003).

For sensory evaluation, meat samples were wrapped in aluminum foil and cooked in a water bath at 100 °C for 30 min (until the internal temperature reached 75 °C). The cooked meat chunks of uniform size were served to semi-trained panelists and evaluated for flavor, juiciness, tenderness and overall acceptability using a 9-point descriptive scale (9=extremely desirable, 1= extremely undesirable).

Statistical Analysis

Experimental results were expressed as Mean±Standard deviation of multiple determinations. The data were analyzed by independent sample *t*- test (P = 0.05). Discriminant function analysis (DFA), a multivariate test was performed to differentiate between the groups using physico-chemical parameters, after the selection of parameters by application of a stepwise discriminant analysis (SDA) using the SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) software package for Windows.

The experimental treatments consisted of combinations of two treatments (with $CaCl_2$ and without $CaCl_2$) and two muscles (breast and thigh), in a total of four groups. The null hypothesis for the equality of the mean vectors for the five parameters in the four tested treatments is H_0: BC=BT=TC=TT. The alternative hypothesis H_a was that, at least one of these mean vectors is different from the others. The resulting subset of step wise discriminant analysis was used in the discriminant analysis to describe differences among groups and observations to groups were allocated. Mahalanobis distance between the two groups was manually calculated using pairwise F-ratios results in below equation (IBM support, 2018)-

$$D_{ab}^2 = [q(N-g)(Na+Nb)]/[(N-q-g+1)\,NaNb] * Fab$$

Where,

a and b - different groups,
q - number of predictors,
N - total number of cases used across all groups,
g - number of groups,
Na - number of cases in group a,
Nb - number of cases in group b, and
Fab - F-statistic (pairwise distances) for comparing groups a and b

Results and Discussion

Physico-chemical Parameters

pH, Water Holding Capacity (WHC), total protein extractability, Myofibrillar fragmentation index (MFI), cooking loss and Shear force value (SFV) are important attributes of meat quality. The effects of $CaCl_2$ on physico-chemical parameters of breast and thigh meat are given in the Table 1.

Table 1: Mean ± SD of variation in physico-chemical parameters of breast and thigh muscles of spent hen on $CaCl_2$ injection (n=12)

Parameters		Breast	Thigh	t-value
pH	Control	5.76±0.025	5.93±0.029	14.832**
	Treatment	5.68±0.032	5.87±0.027	15.738**
	t-value	6.72**	4.61**	
WHC	Control	1.25±0.073	1.44±0.11	4.826**
	Treatment	1.10±0.13	1.27±0.14	2.995**
	t-value	3.185**	3.091**	
Protein Extractability(mg/g)	Control	160.32±9.40	132.51±7.63	7.956**
	Treatment	203.78±10.44	176.11±12.69	3.836**
	t-value	10.71**	6.308**	
Cooking loss (%)	Control	25.77±0.56	22.90±2.34	4.119**
	Treatment	27.82±0.72	25.37±1.12	3.141**
	t-value	2.82**	3.272**	
Shear force value(kgf/cm^2)	Control	3.92±0.63	4.76±0.55	3.441**
	Treatment	3.01±0.25	3.66±0.45	4.266**
	t-value	4.55**	5.299**	
MFI	Control	752.04±15.1	812.86±28.05	6.606**
	Treatment	631.35±9.38	745.70±31.04	12.216**
	t-value	23.45**	5.56**	

***Highly significant (P≤ 0.01)*

In present study, the mean pH of $CaCl_2$ treated (BT and TT) samples were significantly (P<0.01) lower than the respective control (BC and TC) samples. Breast muscles (BC and BT) recorded significantly lower pH than thigh muscles (TC and TT). The lower pH in breast muscles irrespective of treatment may be attributed to the rapid pH decline due to greater proportion of white fibrils in breast muscles (Yu *et al*., 2005). Similar results were reported by Seabra *et al*. (2001) and Chang and Chou (2010) in chicken meat. This significant decrease in pH is mainly due to the production of lactic acid from glycogen (Biswas and Tandon, 2018).

WHC

WHC of the breast meat (BC and BT) samples were found to be significantly (P<0.01) lower than that of the thigh meat samples (TC and TT). Significant (P<0.01) decrease in the WHC of treatment samples (BT and TT) than respective control (BC and TC) samples was observed. According to Bowker and Zhuang (2015), denaturation of sarcoplasmic proteins has more influence on WHC than myofibrillar protein denaturation. The meat quality of both breast and thigh samples irrespective of treatment were noticeably good as WHC values are more than 1 as opined by George *et al*. (1979).

Protein Extractability

Protein extractability is considered to be an important factor influencing protein functionality, such as emulsifying, foaming, and gelling properties (Wang *et al*., 2018). There was a significant (P<0.01) increase in total protein extractability between control and treated samples of breast and thigh muscles. Breast muscles showed

higher($P<0.01$) protein extractability than the thigh muscles in both the control and treatment samples. Increase in protein extractability might be due to early activation of calpain enzyme by $CaCl_2$ treatment leading to proteolysis and enhanced protein extractability (González *et al*., 2012 Wan *et al*., 2018). Similar results were reported by Wang *et al*. (2018) who opined that, increase in the extractability may be due to absorption of Ca^{2+} on the surface of myofibrillar protein. It contributes to the formation of a hydration layer and induction of short-range hydration repulsion between protein molecules, thus improving the functional properties of meat (Wang *et al.*, 2018). The muscle fiber type and the extraction condition also have a large influence on amount and composition of proteins extracted from muscles. (Lan *et al*., 1993).

MFI

The lower MFI value indicates the higher fragmentation of myofiber. In the present study, MFI value significantly($P<0.01$) varied between the muscles and their corresponding treatment groups with lower values in breast muscles and the $CaCl_2$ treatment samples after 24 hr of ageing. According to Cao *et al*. (2012), the combination of conditioning and calcium ion injection is more effective in increasing the action of endogenous proteolytic enzymes in muscle than conditioning alone. MFI is positively correlated with sensory and Warner-Bratzler measure of tenderness and is also related to the degradation of the myofibrillar proteins which may be due to the effects of an endogenous protease i.e. calcium activated factor (CAF) (Olson *et al*., 1976 and 1977).

Cooking Loss

The cooking loss is one of the important concerns in the meat industry. It influences the final yield of product as well as the eating quality of the meat. In the present study cooking loss of thigh meat was found to be significantly ($P<0.01$) lower than the breast meat and the treatment groups (BT and TT) showed higher cooking loss than the control groups (BC and TC). Similar results were reported by Seabra *et al*.(2001). However, Okubanjo *et al*. (2011) reported no significant difference in cooking loss between control and treated ($CaCl_2$) chicken breast samples.

Shear Force Value

The shear force value indicates the tenderness of cooked meat products. In the present study, the mean shear force values were lower in treated samples than respective controls. Injection of $CaCl_2$ has significantly ($P<0.01$) reduced SFV in both breast and thigh samples. Breast muscle (BC and BT) showed significantly ($P<0.01$) lower SFV than thigh muscle (TC and TT). Wan *et al*. (2018) reported that, shear force values decreased with increasing concentration of $CaCl_2$ from 0.1 mol/L to 0.3 mol/L. Similar observations were reported by Okubanjo *et al*. (2011) and they opined that effect of $CaCl_2$ on meat tenderness is probably due to its effect on activation of calpains or through the alteration of protein-to-protein interaction as a result of the elevation of ionic strength. It is also reported that, shear force values were significantly positively correlated with collagen content, yield of muscle and the diameter and area of myofibers and negatively correlated with myofiber density of the muscle (Jaturasitha *et al*., 2008). The difference in SFV observed between the breast and thigh samples may due to the collagen content, which is higher in thigh muscle than in breast meat (Jaturasitha *et al*., 2008; Jeon *et al*., 2010).

Color and Sensory Evaluation

The CIELAB results were presented in Table 2, indicates that breast muscle was lighter ($P<0.01$) in color than thigh muscle. A significant ($P<0.01$) difference was observed for L* and a* values between control and corresponding treatment muscles and no significant ($P>0.05$) difference was observed for b* values between control and treated muscles. The total color differences (ΔE) between BC & BT, TC & TT, BC & TC and BT & TT were 3.71, 3.82, 10.17 and 10.33, respectively. The color differences (ΔE) values were over the JND threshold (2.3 units), which indicates the color can be noticeable by human eye (Sharma, 2003).On the other hand, the results of this study were in agreement with the findings of Perez *et al*. (1998) and Alahakoon *et al*. (2014), who reported a higher lightness in the calcium chloride-marinated meat samples from chicken, horse, cattle and rabbit. Additionally, the lower pH values of treatment samples may have resulted in denaturation of sarcoplasmic and myofibrillar proteins, which may alter their water-holding capacity as suggested by Aktas and Kaya (2001). Therefore, the amount of water dispersed among the muscle fibers could affect the reflectance ability of the meat. The increased lightness caused by calcium chloride treatment was probably due to the more intense disruption of myofibrils because of the activation of calpains by calcium (Aktas and Kaya, 2001).

Table 2: Mean±SD of Hunters color values of breast and thigh muscles of spent hen on $CaCl_2$ injection (n=12)

Parameters/Treatment		Breast	Thigh	t-value
L	Control	55.68±1.89	46.87±2.81	8.987**
	Treatment	59.32±2.25	50.21±2.85	8.672**
	t-value	4.266**	2.880**	
a*	Control	4.11±1.06	6.99±1.72	4.925**
	Treatment	3.71±1.09	6.19±1.67	4.310**
	t-value	0.925	1.154	
b*	Control	12.04±1.73	11.97±1.60	0.102
	Treatment	12.15±1.54	11.11±1.91	1.46
	t-value	0.162	1.193	

***Highly significant (P≤ 0.01)*

The results of sensory attributes of $CaCl_2$ injected and without injected cooked breast and thigh muscles are presented in Fig. 1. BT samples showed higher values for, tenderness, juiciness and overall acceptability followed by TT, BC and TC. However, flavor score did not vary much with injection of $CaCl_2$. Similar results were reported by Wheeler *et al*. (1993) and Lansdell *et al*. (1995). St. Angelo *et al*. (1991) reported that meat infused with 300 mM $CaC1_2$ (10% w/w) had slightly increased bitter and salty flavor without affecting the desirable flavor attributes.

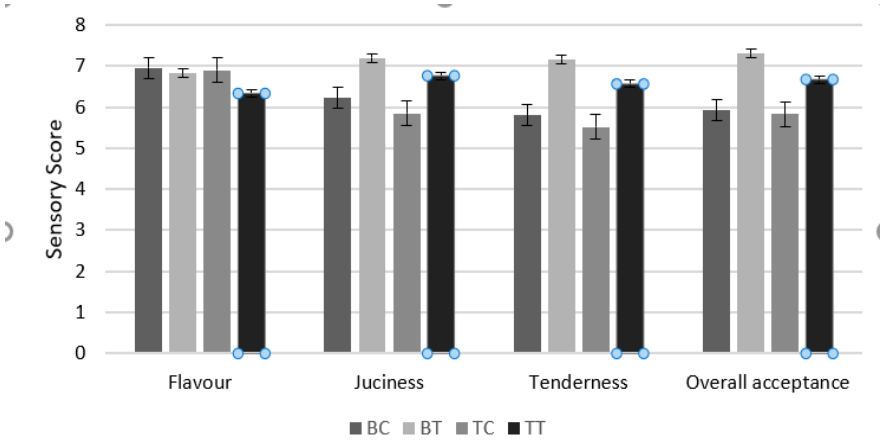

Figure 1: Results of sensory evaluation

Discriminant Function Analysis

Multivariate analyses have been used in different studies related to meat quality. PCA and PLS-DA to detect and quantification of pork meat in other meat by reflectance FT-NIR (Mabood *et al.*, 2020). Pinto *et al.* (2006), studied the performance and carcass traits of two Brazilian chicken using PCA. Discriminant function analysis - multivariate tool was used to evaluate the relative effect of $CaCl_2$ injection on breast and thigh muscles of spent hen. The application of step wise discriminant analysis to the six physico-chemical parameters (pH, WHC, PE, CL, SFV and MFI) of four groups (BC, BT, TC and TT) resulted in the selection of five (pH, WHC, PE, SFV and MFI) parameters. Three discriminant function were developed for each set of data, however only the first two discriminants were found significant and contributed about 99.8 percent to the total variance in physico-chemical parameters. Canonical discriminant analysis was able to differentiate (100 %) all four groups (Fig. 2) using selected physico-chemical parameters. The calculated results of Mahalanobis distances (D^2) are shown in Figure 2.

The D^2 value between BC and BT is lower than TC and TT for physico-chemical which indicates that $CaCl_2$ treatment has comparatively more effect on thigh muscle than breast muscle. These variations in physico-chemical parameters may be due to the concentration of μ-calpain and m-calpain in the different muscles and effect of $CaCl_2$ treatment on accelerating the enzyme activity in respective muscles (González *et al*., 2012; Kripriyalini *et al*., 2017; Wan *et al*., 2018).

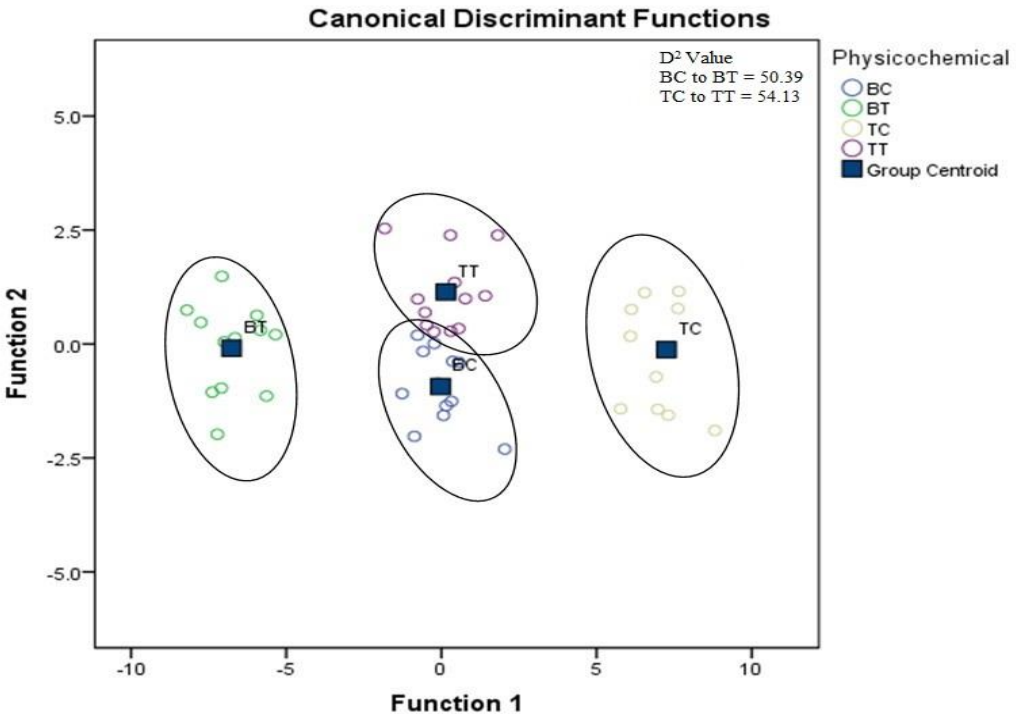

Figure 2: A biplot diagram showing the discrimination of groups on the basis of canonical discriminant analysis of physico-chemical parameters selected by the stepwise discriminant analysis procedure. Mahalanobis distance (D^2) between the two groups are mention in the diagram.

Conclusion

Injecting $CaCl_2$ significantly improves the physico-chemical qualities of both breast as well as thigh muscles. Color of both breast and thigh were influenced by $CaCl_2$ treatment. In addition, significant difference was observed between breast and thigh muscles (both control and treated groups), which may be due to the difference in muscle fibres types. Although, $CaCl_2$ injection has significant effect on both the breast and thigh muscle, multivariate analysis based on the canonical discriminant analysis demonstrated that the effect of $CaCl_2$ is comparatively greater in thigh muscle than breast muscle.

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Conflict of Interests

There is no conflict of interest.

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