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The Effect of High Protein in the Feed Mixture on the Morphological Changes in the Kidneys of Quail Birds

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KEYWORDS

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ABSTRACT: The morphological and biochemical characters and effect of a highly protein diet on Japanese quail were investigated. Total participants in this study were twenty adult male and eighteen female Japanese quail, they were at three different old-stages; 30-days, 90-day and 180-day old. They were offered highly-protein food and water for two weeks. After two weeks, the serum has been centrifugated, hence different indicators including the total protein were assessed. Kidneys were examined with a light microscopy, their masses were estimated. Data analysis was performed using SPSS-22. we observed a decrease in width of middle lobe of kidneys in 90-days old quail birds (22%) and 180-days old quail birds (18%). The perimeter of anterior lobe has been increasing as the age increases, whereas it was a decrease in the middle lobe (35%) and posterior lobe (31%) in 90-days old quail birds. As compared between sex-based groups, it has been shown increase in absolute value of kidney weight among 90-days old (23%) and in 90-days old (7%) female quail birds. Absolute mass has been found more in left kidney at 30-days old (16%) and lower in the right kidney in all age groups female quail birds than that of males. A decrease in length of three lobes of the kidneys was observed in 180-days old females of quail birds (20%), whereas it has been found significantly increase in the width of front lobe (11%) in females quail birds. There was an increase in the amount of general protein and level of albumin in females and males in all age groups. The globin was higher in males than was in females. When comparing the ratio of phosphorus and calcium, it was observed that both of them were higher in females than males. Alkaline phosphate and hemoglobin were elevated in males in 180-days old.

INTRODUCTION

Despite the fact that the terms “feeding” and “fattening” are used interchangeably, they each have a different definition. Feeding is the process of providing or obtaining food for life, growth, production and reproduction, but fattening means overfeeding the animal so that fat accumulates in its muscles in order to improve the quality of the meat and increase its weight [1]. Birds usually starve during settling, hatching or migrating [2]. Or when fattening before slaughter to do meat quality better[3].

It has been observed that vertebrates respond differently to feeding or fattening in birds. For example, small animals and birds can starve during the day, some snakes and frogs can starve for up to two years, and quails can starve for up to 21 days [4]. Actually, the birds, which are endothermic animals and characterized with a higher metabolic rate than mammals, respond to starvation faster and stronger. Thus, the duration of fasting and starvation tolerance in birds are shorter than in mammals of the same size. For example, 31-36 hours in birds is 6 and 3-4 days in mice [5]. Birds, like mammals, go through three metabolic phases during food fasting or starvation to provide sufficient energy to maintain the physiological functions of body systems [1]. The first phase, which is characterized by the utility of carbohydrates and especially liver glycogen as the major origin of energy,

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starts after the last nutrients are absorbed from the small intestine and, as well as fat mobilization and rapid weight loss. In the second stage, after the liver glycogen stores are depleted, the bird oxidizes the accumulated fat, and body weight slowly decreases [6]. In the critical third stage, muscle protein is broken down as fuel after most of the carbohydrates and body fats are exhausted and the bird loses most of its weight and dies quickly if not re-nourished fast.

Weight gain is a frequently documented response in animals and birds to fattening [7]. The increase in the size of the organ is a mechanism in response to the expenditure of additional energy generated in conjunction with physiological functions. However, the rate of organ weight loss depends on the energy consumption of the animals and the method of regulating the use of energy units stored during feeding [1]. The digestive system, due to its excessive energy properties, is the most affected organ in the fattening process. It has been shown that the intestines and liver masses are the organs most consumed in poultry fattening [8,36]. Increase in morphological changes in enterocytes [9,10]. In addition to increased apoptosis [8], histological responses are accepted when chickens are fed. Quail has been proposed as a suitable model for studying biochemical adaptation to short or long term feeding. However, studies of the corresponding histological changes have not been performed. The aim of this study was to study the biochemical and biochemical reactions of male and female domestic Japanese quail after short and long feeding periods (from 30 to 180 days).

The morphological results showed that the bird's kidneys consist of three parts: skull, caudal and medial [11-13]. The results showed that the kidneys consist of two regions: the cortical and medulla oblongata [14,34]. [15] note a pattern of laboratory abnormalities often closely associated with many forms of renal failure in birds.

In the kidneys of a trilobite, the skull is large (length 28 ± 0.15 mm, width 13 ± 0.08 mm), caudal small (length 13 ± 0.07 mm and average lobe 4 ± 0.08 mm) length 30 ± 0.08 mm. and 7.5 ± 0.10 mm, while the current selection criteria for chicks and tails showed that the tail proportions in these birds were significantly higher ($P < 0.01$). [16] showed that the average total kidney length in the turtle was significantly ($p < 0.01$) higher than that in most chickens, which is consistent with the report [17] showing the average total kidney length. The right kidney (60.6 ± 0.047 mm) and the left kidney (60.6 ± 0.081 mm) in the Rhode Island red hen [18] showed that the total length of the kidney in the birds was 7 cm. [16] showed average width of kidney lobes in chickens. Statistically, the mean cranial lobe length (20 ± 0.1 mm) and middle lobe width (5 ± 0.5 mm) were significantly higher than that of the molar (10 ± 0.3 mm). (8.0.2 mm) and chicken (15 ± 0.2) mm) (4 ± 0.4 mm), respectively. The average weight of the kidneys was the heaviest (5.8 ± 0.20 g) and this value was significantly higher for the weight of billionaires (8.9 ± 0.11 g) and chickens (6.8 ± 0.10 g) compared to the weight of the kidneys. The results [19] showed that each kidney consisted of three parts: large skull (average length 28 ± 0.15 mm and width 13 ± 0.08 mm), small caudal (average length 13 ± 0.07 mm and width 4) ± 0.08 mm) and mode. The part (average length 30 ± 0.08 mm in width and 7.5 ± 0.10 mm in width)[16].

MATERIALS AND METHODS

Japanese quail were bred in the Poultry Department of the Agrotechnological Institute of the Peoples' Friendship University of Russia, Moscow, Russia. The experiments were authorized by the Animal Experimental Committee of the Agricultural Institute of Technology in December 2019. In the current experiment, 36 apparently healthy Japanese quails of 30, 60 and 180 days of age were used. They were divided into three groups according to age. The experiment was conducted in February 2020.

For feeding experimental birds, complete synthetic feeds were used: PK-2 (0-30 days), DK-51 (30-44 days), and PK-1P (>44 days). As part of the recipe: wheat, corn, soybeans, sunflower meal, corn gluten feed, vegetable oil, protein concentrate, lysine, methionine, threonine, table salt, monocalcium phosphate, limestone flour, baking soda, complex feed additives for quails . (Vitamins + trace elements) Table 1. Food for quails helps maintain their health at the proper level. This is usually achieved by maintaining a certain level of protein in the diet. Up to 30 days of life, quails need feed mixtures, 26% of which are proteins. For the next two weeks of life, young quails need to be fed less protein - 17%. Adults were given food containing 21% schedule 1 protein.

The composition of the diet provided to the birds is summarized in the study material: Quail birds were fed a diet rich in protein (above the normal rate of 10%) at all stages of the incubation. Protein levels in food were regulated by changes in the composition of the food and therefore in the proportion of proteins: 0-30 days - 28% protein; 31-44 days - 19%; 44 days and more - 24% Table 1. We took three different life stages of birds (30-90-180) days. We measured the weight of a live bird, then killed it, and then studied the morphological changes in the kidneys as a result of a high-protein diet.The experimental diets were corn-soybean meal based and formulated according to nutritional requirements determined by Silva and Costa (J.H.V. Silva, F.G.P. Costa).

Table 1. Composition of the diets provided to the Japanese quail birds.

Quality indicators of complete feed for quail PK-2 (0-30 days)			Quality indicators of complete feed for quail DK-51 (31-44 days)	Quality indicators of complete feed for quails PK-1P (> 44 days)
Name	**Unit rev.**			
Metabolic energy of poultry	kcal / 100g	295	275	285
Crude protein	%	28.0	19,0	24,0
Crude fat	%	3.6	3.5	3,5
Crude fiber	%	4.,8	5.0	5.1
Linoleic acid	%	1.8	1.8	1,8
Lysine	%	1.15	0.85	1.05
Methionine	%	0,55	0.45	0.50
Methionine + cystine	%	0.75	0.70	0.72
Tryptophan	%	0.2	0.2	0.2
Threonine	%	0.65	0.45	0.55
Ca	%	2.3	2.8	3.3
Na	%	0.16	0.16	0,16
P	%	0.7	0.7	0.8
P available	%	0.65	0.53	0.75
Nacl	%	0.33	0.33	0.33

The temperature and day-night cycle light have been adjusted at 20°C and 14 h light/day. The birds were accommodated with food and water. On the day of analysis at 9.00 am, the bird body masses, morphological and biochemical analyses were estimated.

Morphometric analysis: After the birds got the age 30-day, 90-day and 180-day old, just before the birds were sacrificed, we have measured the body mass of bird of each group. Also, The kidneys had been excised to estimate their masses.

To achieve the biochemical analysis, blood samples of all birds of each group were collected after decapitation, the blood were centrifuged by using a Nahita centrifuge model 2698 (GALILEO, Madrid, Spain) at 3000 rpm for 10 min, resulting a clear serum. Directly the serum was stored at –20°C to be used for analyses of different biochemical parameters using commercial kits according to recommendation of the company. The following parameter: total protein, creatinine, urea, triglycerides, cholesterol and High Density Lipoprotein Cholesterol (HDLC) were measured. Using DR-7000D Semi-Automatic Chemistry Analyzer (DIRUI, Changchun, China), all these parameters were determined. Low Density Lipoprotein-Cholesterol (LDLC) was calculated using Friedewald' equation:

LDLC = Total cholesterol-HDL-cholesterol

triglycerides/5

The device used for biochemical examination was ilab 650 (instrument laboratories , USA) diagnostics parameters were Total protein; g l^{-1} – Albumin; g l^{-1} – Globulin; g l^{-1} (Alpha- beta- gamma); Alkaline Phosphates Mm l^{-1}- phosphorous mmol l^{-1}; Ca^{+2} mmol l^{-1}; Erythrocytes 1012/l and Hemoglobin, g l^{-1}.

Statistical analysis: Values were showed as Mean±SEM. The Estimation of significance of difference between groups was done by the one-way analysis of variance (ANOVA). The differences were considered significant when the p-value was > than 0.05.

Following the anatomical dissection of quail, the following morphological changes were studied:

- Measuring the absolute value of the kidneys and both the left and right parts separately;
- The relative weight of both kidneys and the left and right kidneys were calculated separately;
- The total pole length of both kidneys, the three lobes in both left and right kidneys ;
- The total width of the three lobes present in both the right and left kidneys;
- The perimeter of both kidneys together and then measured the perimeter of each kidney alone, the three lobes located in both the right and left kidneys .

RESULTS

Morphological changes of quail bird kidneys

Study of morphological changes in the kidneys depending on the age of birds

We have observed that it was an increase in the lengths of the kidneys depending on age in birds and it has been noticed that the length of middle lobe at ages 30 and 180 days were more increased than the other lobes**.** The total length of the kidneys and their lobes in the quail birds was not significant except for the average lobe of 180 days (P≥0.95) Table 2 .

Table 2. Morphological examinations of kidney in quail**.**

Day	Group			
	Total length (mm)	**Cranial lobe length (mm)**	**Medial lobe length (mm)**	**Caudal lobe length (mm)**
	M ± m	**M ± m**	**M ± m**	**M ± m**
30	43.75 ± 0.95	14.5 ± 0.34	16.17 ± 0.60*	13.08 ± 0.49
90	38 ± 3.18	15.33 ± 0.33	13.67 ± 1.45	9.67 ± 1.76
180	46.43 ± 2.41*	16.29 ± 0.75	17.43 ± 0.75*	12.71 ± 1.13*

In the study, we have observed a decrease in the width of the middle lobe of the kidneys at the age of 90 and 180 days. For the width of the lobes and their surroundings at all ages, the value was insignificant (P≥0.95**)** Table 3 .

Table 3. Morphological results of width kidney lobes in quail birds.

Day	Group		
	Cranial lobe (mm)	**Middle lobe (mm)**	**Caudal lobe (mm)**
	M ± m	**M ± m**	**M ± m**
30	9.5 ± 0.428	9.33 ± 0.667	9.43 ± 0.685
90	8.08 ± 0.611	5.67 ± 0.882*	7.86 ± 0.508
180	8.83 ± 0.70	6.67 ± 1.453 *	8.29 ± 0.522

An increase in the periphery of the kidneys with regard to the cranial lobe with age in addition to a decrease in the periphery of the middle and posterior lobe at the age of 90 days Tables 4 and 5.

Table 4. Morphological changes of the perimeter of kidney lobes in quail birds

Day	Group		
	perimeter of half of the kidney cranial lobe (mm)	**perimeter of half of the kidney, medial lobe (mm)**	**perimeter of half of the kidney caudal lobe (mm)**
	M ± m	**M ± m**	**M ± m**
30	37.5 ± 1.232	45.67 ± 1.453*	33.67 ± 2.390
90	39 ± 3.606	33 ± 5.132	26.67 ± 3.180
180	42 ± 0.756	44.43 ± 1.674*	34.86 ± 1.857

Table 5 . Illustrates total absolute weight of the kidney (g)

Day	M ± m
30	0.74 ± 0.085
90	0.76 ± 0.089
180	0.88 ± 0.166 *

Study of morphological changes in the kidneys depending on the sex of quails

Absolute value of weight increased concomitantly with the age increase of quail birds. In absolute terms, the increase in absolute value was observed among females, especially at the age of 90-180 days. It was found to be high in left kidneys in females aged 30 days while it was low in right kidneys in females. On the 90th day of quail birds, we observed that the absolute value of females was much higher than that of males Table 6.

Table 6. Morphological changes of the absolute mass of kidneys depending on the sex of quail

Parameters	Age Days					
	30		90		180	
	M*	F*	M	F	M	F
Absolute weight (g)	0%	1%	-13%	26%	-26%	20%
Right lobe	1%*	-2%	-12%	23%*	-6%	4%*
Left lobe	-8%	16%*	-11%	23%*	-10%	7%*

* Here and after: M-male, F – female

In our study of the relative mass of kidneys in quail birds, we have noticed a higher value in females than males. We found high relative mass in the right kidneys in males aged 30-180 days and high in left kidneys in females aged 30 days Table 7.

In the table, we noticed a decrease in the lengths of the three lobes of kidneys in female quail birds at 180 days old-age Table 8.

Table 7. Morphological alterations of the relative weight of the kidneys depending on the sex of the quail

Parameters	Age, Days					
	30		90		180	
	M	F	M	F	M	F
Relative weight (%)	10%	30%	-19%	39%	-12%	9%
Right lobe	17%	16%	1%	-3%	23%	-17%
Left lobe	10%	30%	1%	-3%	19%	-14%

Table 8. Morphological length of the kidneys including the length of the three lobes of the kidneys, depending on the sex of the quail

Parameters	Age, Days					
	30		90		180	
	M	F	M	F	M	F
Total Length (mm)	0%	-1%	1%	-2%	0%	-20%*
Cranial lobe (mm)	-1%	2%	-2%	3%	0%	-21%*
Medial lobe (mm)	0%	0%	7%	7%	0%	-19%*
Caudal lobe (mm)	0%	0%	-3%	7%	-1%	-19%*

From our study of kidney lobes, we have found an increase in the width of front lobe of kidney of quail birds in both females and males at the age of 90 days, but birds in the ages of 30-180 days it was noted that in the middle and posterior lobes. With the aging of quail birds we have noticed an increase in the width of the cranial and middle lobes, whereas it was noted a decrease of the posterior lobe in all three stages of age Table 9. The study found that the circumference of the middle and posterior lobes in females at the age of 90 days is significantly higher than other lobes, especially in females.

Table 9. Morphological width of the three lobes of the kidneys depending on the sex of the quail

Parameters	Age, days					
	30		90		180	
	M	F	M	F	M	F
Width of cranial lobe (mm)	-50%	-50%	-6%	11%*	-7%	-22%
Width of medial lobe (mm)	-51%	-38%	-12%	31%*	-4%	-31%
Width of caudal lobe (mm)	-39%	-38%	-15%	31%*	-2%	-31%

DISCUSSION

It was found that the length of the kidney increases with age, and this result is similar to the results obtained by [20] By comparing renal lobes by length, the cranial lobe was found to be the longest, followed by the middle, then the caudal, and this result was identical to the results of the study by [16] However, in another study by Bath, it was found that the middle lobe is the longest between the lobes, cranial and caudal. In the course of the study, we found that the length of the kidney was greater than its width, but this result was opposite to the findings of Kumar The study found that the cranial lobe of the kidneys in quail is longer than in ducks, but shorter than in mallard and chickens [16] The width of the average quail share was greater than that of the mallard, duck, and chicken [16]. We noticed that kidney weight increases with age Table 5, and these results are consistent with those from a study in Zimbabwe [20]. The total weight of quail is less than that observed in the results [16] in other birds. The weight of the kidneys in females is higher than in males, and this is due to the fact that the kidneys of females contain a greater number of nephrons and are larger in diameter, therefore, the glomerular filtration rate in females becomes lower than in males [19, 21].

The average body weight of quail females (209 ± 18.737) was twice as high as that of males (179 ± 11.665) [22, 23].

Comparisons of the biochemical values of females and males of quails at the age (1-3-6) are presented in Tables 10 and 11. The values of the following parameters total protein, albumin, globulin, calcium, phosphorus, and erythrocytes have been increased with age (by months)[23,25,35].

The current study found that quail fed a higher diet increased body weight by about **23%**. Weight gain from complementary feeding has been described in various complementary feeding bird species such as quail, turkey, and sparrows. The rate of weight gain depends on many factors, including gender, bird species, and initial body weight. The current study showed a significant increase in the measured mass of the kidney lobes. A similar result has been reported in sparrows and migrating blackcaps. This is consistent with the fact that the renal system requires a lot of energy and building units to perform its function. An increase in kidney mass may be a reactive mechanism for increasing energy intake during complementary feeding [26]. The increase in lobe length reported in this study is likely responsible for the increase in kidney mass. An increase in the length of the kidney lobes, together with an increase in the absolute weight of the kidney, definitely increases the functional capacity of other systems and thus leads to a greater increase in body weight in subsequent stages. According to the current study, turkey supplementation showed a 30% accumulation in the kidney lobes and a 20% increase in relative weight.

The second largest biochemical changes were noted in the blood, where total protein showed increased changes indicative of regenerated muscle and vital organ building.

Due to the high protein concentration, the cofactors involved in the absorption of amino acids from the intestine were activated [8].

Serum biochemical analysis showed increased total protein levels with high protein feeding. This result is consistent with the high protein diet of ducklings. The increase in albumin and globulin levels is likely associated with increased biosynthesis or decreased degradation.

This study found that the levels of albumin, globulin, and total protein were higher in females compared to males. These differences can be explained by physiological changes in female obesity as a result of oviposition [27]. During implantation (implantation period), the synthesis of triglycerides, phospholipids and cholesterol in the liver increases. These fats make up lipoproteins, which circulate in the blood and go to the ovaries. Consequently, females have higher triglyceride and cholesterol levels than males. In addition, in females of other species, there was a noticeable increase in the concentration of total protein during oviposition [28]. This is associated with an increase in the secretion of estrogens, which leads to an increase in the production of the precursor vitellinogenin and yolk lipoproteins [29], which leads to an increase in the total concentration of these proteins in the blood and blood serum. proteins. birds. Higher total protein concentrations in laying birds are associated with marked increases in serum albumin and globulin levels [30].

Comparing the increase in total protein at 6 months of age in male and female quails, it was found that it was higher in males than in females. For albumin, contrary to expectations, it was higher in females (12.65) than in males (12.09) at the age of 6 months [31]. At this age (6 months), the proportion of globulin in males and females of quail is the same -21.15, while the A / G value in males (1.75) and in females (1.67). In addition to this, an increase in hemoglobin was found at 3 months of age among females and males. Globulin levels in males were higher than in females due to the high protein content of the feed provided to quails [32]. Alpha globulin was higher in males at 1 month, while in females at 3 months, and beta globulin levels were high in males and females at 6 months. At the age of 3 months, gamma globulin was increased in males, and at 6 months in females.

The increase in total protein and albumin may be associated with estrogen secretion early in oviposition [28].

Alkaline phosphate in males was high at 3 months of age, while in females it was high at 1 month of age, and when comparing alkaline phosphate in females and males, it was the same for both sexes [31]. Phosphorus was high in males at 6 months of age and high in females at 1 month of age. However, when comparing the indicators of phosphorus in females and males, it was found that the phosphorus content in females is higher than in males. The percentage of calcium was high in males at 1 month of age, while in females at 6 months of age, and it was observed that an overall high level of calcium was found in females and males at all ages studied. Blood calcium levels in the current study are consistent with observations made by [33]. It has been suggested that calcium levels can be attributed to increased secretion of steroid hormones. However, when comparing erythrocyte counts between females and males, there were no differences between them; females and males had an increased number of erythrocytes at the age of 6 months [31]. In the study of hemoglobin, it was higher in males than in women [31], and increased in males at 3 months, and in females at 1 month.

Table 10. Biochemical parameters of the blood of quails (males)

№ п/п	Indicators	1	3	6
1	Total protein, g l^{-1}	35,01 ± 0,05	32,03 ± 0,04	36,08 ± 0,04*
2	Albumin, g l^{-1}	12,22 ± 0,06*	11,39 ± 0,08	12,09 ± 0,06
3	Globulin, g l^{-1}	22,70 ± 0,03	23,79 ± 0,09*	21,15 ± 0,05
4	- Alpha	2,83 ± 0,05*	2,74 ± 0,03	2,55 ± 0,08
5	- beta	5,19 ± 0,05	5,09 ± 0,05	6,09 ± 0,04*
6	- gamma	15,87 ± 0,04	15,96 ± 0,06*	15,02 ± 0,03
7	Alkaline Phosphates, Mm l^{-1}	4,65 ± 0,30	4,71 ± 0,33*	4,15 ± 0,30
8	phosphorous, mmol l^{-1}	2,23 ± 0,02	2,07 ± 0,08	2,52± 0,02*
9	potassium, mmol l^{-1}	3,52 ± 0,10*	3,48 ± 0,15	3,23 ± 0,15
10	Erythrocytes, 10^{12}/l	3,42 ± 0,05	3,25 ± 0,02	4,05 ± 0,05*
11	Hemoglobin, g l^{-1}	130,50 ± 0,10	132,41 ± 0,13*	128,45 ± 0,19

Table 11. Biochemical parameters of the blood of quails (females)

№ п/п	indicator	1	3	6
1	Total protein, g l^{-1}	29,75 ± 0,04	31,03 ± 0,05	34,05 ± 0,04*
2	Albumin, g l^{-1}	14,22 ± 0,03*	11,55 ± 0,08	12,65 ± 0,06
3	Globulin, g l^{-1}	20,10 ± 0,05	21,68 ± 0,06*	21,15 ± 0,04
4	- Alpha	2,56 ± 0,04	2,64 ± 0,06*	2,57 ± 0,08
5	- beta	5,35 ± 0,15	5,43 ± 0,05	6,32 ± 0,06*
6	- gamma	14,45 ± 0,05	15,05 ± 0,06	15,09 ± 0,03*
7	Alkaline Phosphates, Mm l^{-1}	4,75 ± 0,20*	4,65 ± 0,33	4,15 ± 0,20
8	ph+3, mmol l^{-1}	2,63 ± 0,02*	2,56 ± 0,08	2,58± 0,02
9	Ca^{+2}, mmol l^{-1}	3,59 ± 0,10	3,63 ± 0,14	4,03 ± 0,10*
10	Erythrocytes 10^{12}/l	3,51 ± 0,03	3,29 ± 0,02	4,00 ± 0,16*
11	Hemoglobin, g l^{-1}	131,50 ± 0,15*	131,45 ± 0,13	129,03 ± 0,12

CONCLUSIONS

The kidneys are the means by which the metabolic diseases can be diagnosed, as we noted from the study the effect of the diet on the morphological changes in the kidneys in the kidneys of quail birds, as well as its association with sex through the work we have done.

We have found that many of the parameters evaluated differ significantly between the sexes. Therefore the sex effect should be considered to avoid unwanted sources of variation and hence miscalculation of at least some blood parameters. Moreover, other factors, such as daily variation and treatment of birds, should also be taken into account, as they may affect different criteria. Bird capture and seizures may also alter some variables.

Conflict of interest

The authors declare no conflict of interest.

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