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Probiotics in Industrial Poultry Farming

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Abstract: In the conditions of industrial production, the problem of growing poultry with increased resistance to various diseases is relevant because the birds are exposed to various stress conditions. Traditionally, disease prevention is carried out through the use of vaccines, chemical pharmaceuticals, and antibiotics. The global use of therapeutic drugs contributes to the emergence of resistant strains of microorganisms and a decrease in the effectiveness of treatment; some of them have an immunosuppressive effect. The article presents the results of research on the effect of the preparation "*Lactobacillus plantarum*, *Enterococcus faecalis*, and *Propionibacterium freudenreichii* ssp." on the immune status, growth rate, and safety of experimental stock in broiler chickens. Studies were carried out on 150 one-day-old Ross-308 broiler chicks that were divided into three groups of 50 birds. The control Group (G3) was fed a standard ration based on the phases of growth, while the experimental Groups (G1 and G2) were supplemented with probiotics at different doses in the feed. The experiment was designed to last for 41 days. In the 3rd week, the average live weights of G1 and G2 broiler chickens exceeded those of G3 by 5.5 and 4.1%, respectively. In the 6th week, G1 and G2 broiler chicks had average live weights that exceeded those of G3 by 7.7 and 7.4%, respectively. The survival rates of the flocks in G1 and G2 were 4 and 2% higher, respectively than in G3. The average titers for Newcastle Disease and infectious bronchitis viruses in G1 were 314.9 and 53.5% higher, respectively than in G3, while in G2 they were 3.7 and 21.8% lower, respectively. For infectious bursal disease, G1 and G2 exceeded the control group by 74.2 and 6.4%, respectively. The meat yield of G1 and G2 was higher at 17.2 and 9.1%, respectively, compared to G3. Probiotics have shown many beneficial properties, including the ability to improve immunity, gut structure, and gut barrier function in broilers. These factors can improve digestion and absorption, ultimately increasing the quality and safety of products.

Keywords: Probiotic, Broiler Chickens, Immune Status, Growth Rate, Industrial Poultry

Introduction

The use of antibiotics to maintain animal welfare, promote growth and enhance productivity has been practiced for over 50 years. However, as early as the 1950s, researchers expressed concern about the development of antibiotic-resistant bacteria. The use of feed additives has contributed to the success achieved in current broiler production. Feed additives are generally considered to be materials used to increase the effectiveness of nutrients and have an effect on improving poultry performance (Farag and Alagawany, 2019; Saeed *et al*., 2019). Several feed additives are used in poultry feed, such as antibiotics, probiotics, oligosaccharides, enzymes, and organic acids. They are included in the diet of poultry and animals to promote growth due to their potential effect in increasing feed intake. In addition, low levels of additives in poultry feed may increase the production of avian protein for human consumption, which in some cases may reduce the cost of livestock and poultry production. Traditionally, disease prevention is carried out through the use of vaccines, chemical pharmaceuticals, and antibiotics. The global use of therapeutic drugs contributes to the emergence of resistant strains of microorganisms and a decrease in the effectiveness of treatment; some of them have an immunosuppressive effect. The introduction of a vaccine to poultry with a low level of resistance does not lead to the development of a full-fledged immune response and is accompanied by an increase in the number of post-vaccination complications. Subtherapeutic antibiotics are commonly used to prevent disease and increase body weight (Bai *et al*., 2017; Gong *et al*., 2018; Abd El-Hack *et al*., 2021; Kang *et al*., 2021). Some consumers have a negative attitude toward subtherapeutic antibiotics. This is due to the growing evidence that antibiotic resistance genes can be transmitted from animals to humans. Immunodeficiencies in young birds are accompanied by disruption of the normal designation of the intestinal microflora. Probiotics are recommended for the prevention of dysbiosis. Probiotics have demonstrated many beneficial properties, including the ability to improve immunity, gut structure, and gut barrier function in broilers. Probiotics promote the development of beneficial microflora, increase productivity and improve body resistance. These factors can improve digestion and absorption, which can ultimately increase the production of quality and safe products (Richards *et al*., 2005; Glaskovich *et al*., 2012; Haustov *et al*., 2017; Lysko, 2020; Meher *et al*., 2021). The addition of probiotics to flock diets can not only improve the production and organoleptic quality of poultry and poultry products but also reduce an environmental load of raising poultry. Thus, the retention of nutrients in the diet through the addition of probiotics represents a potential opportunity to improve growth performance and reduce the environmental load (pollutants) from poultry production (Chervonova, 2017; Ovcharova and Petrakov, 2018; Swaggerty *et al*., 2019; El Jeni *et al*., 2021; Petrone-Garcia *et al*., 2021). Currently, side effects from the use of probiotic preparations in poultry farming have not been established. By incorporating recombinant *Lactobacillus* cultures in poultry diets, broilers have gained significantly in weight, resulting in lower production costs and reduced environmental impact (Rinttilä and Apajalahti, 2013; Lantseva *et al*., 2015; Temiraev *et al*., 2016; Lin *et al*., 2022). Not only can probiotics reduce nutrient requirements by increasing nitrogen and phosphorus utilization, but some probiotics have also demonstrated significant immunomodulatory potential (Eeckhaut *et al*., 2016; Bilal *et al*., 2021; Khodorovich, 2021; Sarba *et al*., 2021). Protection against pathogens and facilitation of digestion and nutrient utilization can be addressed by modulating the immune response (Askelson *et al*., 2014; Gong *et al*., 2018; He *et al*., 2019). These benefits can be achieved by enhancing the innate and acquired immunity of poultry. However, more research is needed to identify such differences.

Probiotics can be used instead of antibiotics, as they produce substances with antibacterial activity. They also contribute to the development of beneficial microflora, increase productivity and improve the body's resistance.

The purpose of the research is to study the effect of the experimental drug "*L. plantarum, E. faecalis,* and *P. freudenreichii* ssp." on the immune status of animals, growth rate, and safety of the experimental livestock. To achieve this goal, the following tasks were set: To analyze the growth rate of broiler chickens; to study the effect of the drug on the safety of livestock; to study the effect of the drug on the intensity of immunity against viral diseases; to calculate the economic efficiency of using the drug in livestock rations.

Materials and Methods

The study protocol was discussed and approved at a meeting of the local ethical committee of the Department of Veterinary Medicine and Biotechnology of the Kuzbass State Agricultural Academy of the Ministry of Agriculture of the Russian Federation on September 1, 2022.

A total of 150 Ross-308 one-day-old broiler chicks were received and divided randomly into three groups of 50 chicks. The experimental broilers were treated concerning the provisions of the "Methodology for scientific and industrial research on the feeding of poultry" (Fisinin, 2013) were taken into account. The experiment was conducted at LLC Poultry Farm "Trudarmeyskaya" of the Prokopievsk district of the Kemerovo region.

Chickens of the control group were fed standard ration supplemented with antibiotics according to rearing phases. The main diet is presented in Table 1.

The experimental groups (G1 and G2) were fed an experimental batch of probiotic "*L. plantarum*, *E. faecalis*, *P. freudenreichii* ssp." in different doses of mixed fodder weight according to the scheme of the experiment (Table 2). The experimental preparation "*L. plantarum, E. faecalis* and *P. freudenreichii* ssp." is an association of deposited certified probiotic strains of *Lactobacillus plantarum* B-11007, *Enterococcus faecalis* H/22 and *Propionibacterium freudenreichii* B-6561 FZ, purchased from the Kurchatov Institute (Russia). The concentration of the probiotic was selected empirically by an adequate level of consumption of probiotic microorganisms.

The experimental preparation contains strains of microorganisms *L. plantarum*, *E. faecalis* and *P. freudenreichii* ssp. The preparations were injected into water daily, calculating the rate of the input based on actual feed consumption by poultry. The preparation was applied using the drinking method.

The feed was distributed manually after preliminary mixing the probiotic with mixed feed. The poultry was kept under floor-rearing conditions. The microclimate parameters during the period of research were following the norms specified in the MPC (Maximum Permissible Concentration), as well as those established at the enterprise as a whole. The main indicators were recorded by a computer.

Water supply was provided from underground sources. Water was pumped to water towers. Water for drinking animals was clear, colorless, and without foreign smell and taste. The farm implemented nipple watering of poultry, both young and adult birds. It was carried out by adjusting the height of the structure; the nipples used were by PKN-6 (China). Day-old chicks were offered water heated to 30°. From a week of age, the temperature should not be lower than 25°. After 21 days of life, it was reduced to 17-19°.

Feeding was carried out using FLUXX 360 feeders (Big Dutchman, Russia). For young animals, bunker feeders KB-2 or KB-5 (Russia) were used. Delivery and distribution of feed were carried out using a feed truck ZSK-10 (ZiL, Russia). All processes that ensure the life of poultry were automated. For high productivity, it is necessary to provide poultry with high-quality feed and water on time and create the most comfortable conditions. One of the most important factors for successful production is the creation of an optimal microclimate for growing poultry.

Air ventilation was carried out using tunnel ventilation. The hoods were located in the end walls, which provided the broilers with an optimal microclimate. The premises were heated using a boiler house, which was located on the territory of the poultry farm. It provided uninterrupted heating and a supply of warm water to the poultry drinking bowls. The lighting in the chicken coop corresponded to the required parameters for a given poultry keeping and was provided by incandescent lamps installed in the ceiling of the poultry house.

To ensure epizootic well-being, a well-executed system of preventive measures is required; territorial separation of technological production links, the creation of a favorable microclimate in poultry premises, advanced technology, and a strict veterinary and sanitary regime are necessary. The territory of the poultry farm was fenced and divided into zones: Hatchery and poultry houses. All shops (adult hens, parent flock, slaughter shop, mechanical workshops, laboratories, feed shop, egg store) were somewhat removed from each other. The poultry farm strictly observed the regime of a closed economic entity.

Table 1: Diet of broiler chickens, %

Indicator	Age, days		
	1-14	15-29	30-41
Corn	33.20	37.90	39.60
Wheat	24.60	23.10	23.10
Soybean meal	22.00	20.00	20.00
Sunflower oil	3.00	4.00	4.50
Fish meal	8.00	6.00	4.00
Premix	1.00	1.00	1.00
Salt	0.20	0.20	0.20
Corn gluten	6.00	6.00	5.50
Tricalcium phosphate	1.00	1.50	2.10
Limestone	1.00	0.30	0.00
Total	100.00	100.00	100.00
100 g of compound feed contains, %			
Metabolic energy, kcal	310.70	318.10	321.80
Crude protein	22.50	20.70	19.30
Crude fiber	3.10	3.00	3.00
Calcium	1.10	0.95	0.94
Total phosphorus	0.70	0.71	07.00
Available phosphorus	0.42	0.43	0.45
Sodium	0.23	0.20	0.17
Lysine with additive	1.36	0.30	1.14
Methionine + cysteine with add	0.98	1.00	0.93
Linoleic acid	3.35	3.90	4.20
Tryptophan	0.26	0.23	0.22

Table 2: Experiment scheme

Group	The special feature of feeding
G3: Control group	Complete Feed by rearing phase (CF) + antibiotics (days 2-6, 20-23)
G1: Experimental group 1	CF + probiotic at a dose of 0.15% of the feed weight daily
G2: Experimental group 2	CF + probiotic at a dose of 0.3% of the feed weight daily

The duration of the experiment was 41 days. To establish the effect of probiotic preparation "*L. plantarum*, *E. faecalis* and *P. freudenreichii* ssp." on the experimental broiler, the following indicators were taken into account: Average daily live weight, absolute live weight gain, feed expenses per 1 kg of gain and safety. The main productive qualities of broiler chickens were determined following the requirements of the "Methodology for scientific and industrial research on the feeding of poultry" (Fisinin, 2013).

To study the effect of the probiotic preparations on postvaccination immunity intensity, the blood samples were assessed by Enzyme Immunoassay (ELISA) and hemagglutination inhibition reaction (RTGA). Blood was taken from the broilers at the age of 41 days from the axillary vein in the morning in the standard way. A total of 75 blood serum samples were taken, 25 samples from each group.

To establish the effect of the probiotic preparation of the experimental population of broiler chickens of the Roos-308 cross, in the research, the live weight of broiler chickens was determined (using the method of individual weighing weekly), the safety of the livestock (daily) and the intensity of immunity to viral diseases.

At the end of the experimental period, when growing broiler chickens, we considered the intensity of immunity. The following sets were used for the analysis:

- To determine antibodies to the infectious bursal disease virus by enzyme immunoassay when testing sera in one dilution (Federal Center for Animal Health, Russia)
- To detect antibodies to the Newcastle disease virus in the hemagglutination inhibition test (Federal Center for Animal Health, Russia)
- To determine antibodies to the chicken infectious bronchitis virus by enzyme immunoassay when testing sera in one dilution (Federal Center for Animal Health, Russia)

The ELISA results were recorded after stopping the reaction using a Thermo Scientific Multiskan FC microplate photometer (Thermo Fisher Scientific, Finland) with a vertical beam of light at a wavelength of 405 nm.

Statistical data processing was carried out on a personal computer using Microsoft Office Excel with confirmation of reliability with Student's t-test in the following values: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Results

The effectiveness of the probiotics "*L. plantarum, E. faecalis,* and *P. freudenreichii* ssp." in broiler chicken diets were studied in our research experiment.

The dynamics of the live weight of broiler chickens when the probiotic " *L. plantarum*, *E. faecalis*, and *P. freudenreichii* ssp." was included in the diet is shown in Table 3.

By the 3rd week, broiler chickens supplemented with" *L. plantarum*, *E. faecalis*, and *P. freudenreichii* ssp." achieved an average live weight of 947 g in G1, which was 49.6 g (5.5%) higher than the control, while in G2 was 36.6 g (4.1%) higher than the control.

It is important to note that the predominant increase in live weights was consistent in the experimental groups. In the 6th week, G1 achieved an average live weight of 3005.1 g, which was 215.1 g (7.7%) higher than G3. The G2 broiler chickens achieved an average live weight of 2997.1 g (7.4%) more than G3. According to the results, it can be seen that the effectiveness of the probiotic dose is evident in G1.

The growth rate and survival of experimental broiler chickens are shown in Table 4. The average daily gain in G1 was reliably higher than G3 by 5.3 g (7.9%), while in G2 it was higher by 5.1 g (7.6%).

The absolute gain of G1 broiler chickens was reliably higher than G3 by 214.9 g (7.8%), while in G2 was 106.8 g (3.9%).

The relative gain of G1 broiler chickens was 485,6% higher than the control group and 422% higher in G2. This is explained by the fact that the mechanism of action of probiotics "*L. plantarum, E. faecalis, and P. freudenreichii* ssp." is to improve the digestibility and absorbability of nutrients, thereby increasing the weight gain.

Retention of poultry is an important indicator in industrial production, it directly affects economic efficiency, as well as the study of the effects of various preparations and additives. Mortalities were 8, 4, and 6% in the control, G1, and G2, respectively. The high percentage of preservation of broilers in the experimental groups indicated a decrease in morbidity and increased resistance to macroclimatic factors due to the action of probiotic supplements.

Throughout the experimental period, the feed intake of broiler chickens was recorded as shown in Table 5. The experimental birds had a pronounced appetite with increased feed intake and probiotic supplementation. In terms of feed intake, the experimental groups outperformed the control group due to the high growth energy of the chicks contributing to increased live weight gain. We did not observe any rejection of feed.

For the whole period of the experiment, broiler chickens fed with the probiotic supplement "*L. plantarum, E. faecalis,* and *P. freudenreichii* ssp." in G1 and G2 consumed on average 4,990 and 4,990 g, respectively of mixed fodder. The control group consumed 5,100 g of mixed fodder, which was 110 g (2.1%) more than G1 and G2, while the growth rate in G3 (Control) was significantly lower.

Adding probiotics to the basic diet stimulated metabolic processes and increased the growth intensity and live weight based on increased feed conversion with reduced production cost. On average 1.82 kg per kilo of body weight gain in broiler chickens fed the basic ration in contrast to G1 and G2 where the feed consumption per kilo of gain was 1.66 kg.

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The effect of probiotics "*L. plantarum, E. faecalis, and P. freudenreichii* ssp." on the post-vaccination immunity to Newcastle disease virus, infectious bronchitis, and infectious bursal disease was carried out by analyzing blood sera that were collected from the wing vein at 41 days. The antibody titers of broiler chickens are shown in Table 6. The results showed that the control group had a low level of post-vaccination antibodies to the Newcastle disease virus, infectious bronchitis, and infectious bursal disease.

G1 revealed an average antibody titer to Newcastle Disease virus (ND) up to 47,360 units (314.9%) higher than in G3 and in G2 up to 0.560 units (3.7%) lower. For Infectious Bronchitis (IBD), the results revealed average titers that were higher by 1,812.24 units (53.5%) in G1 and by 739.28 units (21.8%) in G2 compared to the control. For Infectious Bursal Disease (IBD), G1 exceeded the control by 2,366.6 units (74.2%) and G2 by 205.08 units (6.4%).

Based on the results obtained, we can conclude that the experimental chickens that received probiotics had a more pronounced level of antibody titers.

Newcastle Disease Virus Antibody titer revealed that the birds are immune with an immunization efficiency of 80% or more. Infectious Bursal Disease and Infectious Bronchitis Viruses revealed that broilers are immuned at a rate of 90% or more. The post-vaccination immunity status of broiler chickens is shown in Table 7. G1 and G1 revealed that immunity was tense to all viral diseases.

Thus, the use of probiotics positively affects the intensity of immunity of poultry and the more pronounced production of antibodies to viral diseases after the use of vaccines in broiler chickens.

Table 3: Dynamics of growth of live weight of broiler chickens, g

Indicator		Groups	
Age, weeks	G3: Control	G1	G2
Daily allowance	44.3±00.40	44.5±00.4	44.6±00.3
1	186.2±03.78	188.5±02.6	188.2±02.8
2	474.0±07.20	482.0±12.1	481.0±10.4
3	897.4±10.40	947.0±10.0***	934.0±09.6*
4	1465.8±12.10	1571.0±11.7***	1511.0±08.4**
5	2145.0±19.30	2323.0±19.8***	2309.0±16.9***
6	2790.0±23.20	3005.1±21.2***	2997.1±19.7***
In % compared to the control group	100.0	7.7	7.4

Note: Hereinafter, the difference is significant at *p<0.95 -**p>0.99 -***p>0.999: - *p<0.95 -**p>0.99 -***p>0.999

Table 4: Growth rate and survival of experimental broiler chickens

Group	Average daily gain, g	Absolute increase, g	Relative increase, %	Conservation, %
G3: Control group	66.90±0.84	2,745.7±16.1	6,197.9	92
G1: Experimental group 1	72.20±0.87***	2,960.6±13.4***	6,683.5	96
G2: Experimental group 2	72.00±0.70***	2,852.5±12.6***	6,619.9	94

Table 5: Feed consumption during rearing

	Groups		
Indicator	G3: Control	G1	G2
Feed intake during the growing period per head, g	5,100	4,990	4,990
Feed consumption per 1 kg of growth, kg	1.820	1.660	1.660

Table 6: Indicators of antibody titers in broiler chickens

Diseases	G3: Control	G1	G2
Newcastle disease virus	15,040±3,503	62,400±13,831***	14,480±2,696
Infectious bronchitis	3384,640±495,274	5196,880±455,255*	4123,920±462,469
Infectious bursal disease	3191,200±481,206	5558,160±551,334***	3396,280±491,871

Table 7: The tension of post-vaccination immunity in broiler chickens

	Immunity tensions, %		
Disease	G3: Control	G1	G2
Newcastle disease virus	64	92	80
Infectious bronchitis	80	94	88
Infectious bursal disease	76	100	96

The economic efficiency of the probiotics used helped to establish a more profitable use for the enterprise. The carcass slaughter yield in G1 and G2 were higher than G3 by 215.1 g (7.7%) and 207.1 g (7.4%) respectively. The inclusion of probiotic preparations "*L. plantarum, E. faecalis* and *P. freudenreichii* ssp." in the basic diet of broiler chickens contributed to 126.72 and 117.97 kg of meat yield in G1 and G2 which were 18.62 kg (17.2%) and 9.87 kg (9.1%) higher than G3.

Additional profit in G1 and G2 was 1,712.76 and 535.36 roubles, respectively. When probiotic supplement was used, a high level of profitability of meat production in broilers was achieved.

Discussion

The use of the preparation " *L. plantarum, E. faecalis*, and *P. freudenreichii* ssp." in broiler chickens had a positive effect on the growth and safety indicators. The high percentage of retention in the experimental groups indicated a reduction in morbidities and increased resistance to macroclimatic factors.

The supplemented birds had a more pronounced level of antibody titers. According to our results, G1 and G2 revealed strained immunity to all viral diseases. This indicated that the application of probiotics positively affects the immunity levels of poultry and a more pronounced production of antibodies against viral diseases after the vaccination act.

The results indicated that the inclusion of the probiotic preparation "*L. plantarum, E. faecalis* and *P. freudenreichii* ssp." in the broiler's diet contributed to the maximum productivity.

The results of our study coincide with the results of several authors who confirmed that the use of probiotic preparations has a beneficial effect on the immune status (Glaskovich *et al*., 2012; Attia *et al*., 2020; Lysko, 2020; Kang *et al*., 2021) and productivity of poultry (Rinttilä and Apajalahti, 2013; Lantseva *et al*., 2015; Temiraev *et al*., 2016; Bai *et al*., 2017) and in the future can serve as an alternative to the use of subtherapeutic antibiotics (He *et al*., 2019; Swaggerty *et al*., 2019; Bilal *et al*., 2021; Meher *et al*., 2021).

In the works of the authors, an assessment is made of the effect of various probiotic preparations and their strains on productive indicators, as well as indicators characterizing the state of poultry health. Probiotics may reduce nutrient requirements by increasing nitrogen and phosphorus utilization, but some probiotics have also shown significant immunomodulatory potential (El Jeni *et al*., 2021). Defense against pathogens and facilitation of digestion and nutrient utilization can be addressed by modulating the immune response (Rinttilä and Apajalahti, 2013). These benefits can be achieved by enhancing the innate and adaptive immunity of poultry. In particular, Swaggerty *et al*. (2019) suggest that influencing innate immunity by modulating the proliferation of macrophages, heterophils, and B1-type lymphocytes is more beneficial than stimulating acquired immunity.

Many of these benefits are due to the supposed modification of the intestinal ecosystem through the biological effects of probiotics. The impact of probiotics is highly dependent on several parameters, including the strains of microorganisms used, the concentration of probiotics in the feed, the interaction of probiotics with individual components of the diet, the interaction with the local microbiota, the age of the broilers and the nutritional and health status of the poultry. We also noted a similar positive effect when studying a new probiotic preparation "*L. plantarum*, *E. faecalis* and *P. freudenreichii* ssp.", which allows us to conclude its promising use in poultry farming and the possibility of replacing subtherapeutic antibiotics with it.

Conclusion

The novelty of the experiment is associated with the study of the effectiveness of the new probiotic preparation "*L. plantarum*, *E. faecalis* and *P. freudenreichii* ssp." on the wholesale livestock of poultry and the possibility of using a probiotic preparation as an alternative to feed antibiotics used in the industrial cultivation of broiler chickens. Evaluation of the study results showed a positive effect of the considered drug on the experimental poultry population.

By the 3rd week, the average live weight of G1 and G2 exceeded that of the control group by 5.5 and 4.1%, respectively. In the 6th week, the G1 and G2 broiler chicks exceeded the control group by 7.7 and 7.4% respectively in average live weight. The survival rate of the flock in G1 and G2 was 4 and 2% higher than that of the control group.

The average titer for Newcastle Disease (NB) in G1 was 314.9% higher and in G2 was 3.7% lower compared to the control. The average antibody titer for Infectious Bronchitis Virus (IBD) prevailed in G1 and G2 by 53.5 and 21.8% respectively compared to the control group. For Infectious Bursal Disease (IBD) antibody titer, G1 and G2 exceeded the control group by 74,2 and 6,4%, respectively.

Inclusion of probiotic preparations "*L. plantarum*, *E. faecalis* and *P. freudenreichii* ssp." in the basic diet of broiler chickens allowed to obtain meat yield of p to 17.2 and 9.1% in G1 and G2, respectively higher than the control group. Additional profit in G1 and G2 was 1712,76 and 535,36 roubles, respectively. When probiotic supplement was used, a high level of profitability in broiler meat production was achieved.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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