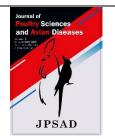
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Effects of Rosemary (*Rosmarinus officinalis L.*), Onion (*Allium cepa L.*) Extracts as Feed Additive on Layers Performance, Hematological and Blood Biochemical Parameters of White Leghorn

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ABSTRACT

This study evaluated the effects of rosemary and onion extracts on leave performances and some hematological and serum biochemical parameters of White Leghorn layers at Haramaya University Poultry Farm. One hundred twenty (120) White Leghorn layers at thirty-two weeks of age were randomly allocated to four treatments, each replicated three times with ten layers and one cock per replication and managed on a deep litter system for 70 days. The treatments were T1 (control group without any addition of feed additives), T2 (water with 4 milliliters of onion extract), T3 (water with 4 milliliters of rosemary extract), and T4 (water with 4 milliliters of onion and rosemary extract mixture). Body weight was taken at the beginning and end of the experiment to determine body weight change. Feed intake was determined as the difference between the feed offered and refused divided by the total number of layers in the replication. Data were subjected to be analyzed by SAS software version 9.4 (SAS, 2019), and the significance in treatment mean was determined by the least significance difference (LSD) for all parameters considered. The daily feed intake of chickens in T2 (97.59 \pm 0.72g) was significantly (p<0.01) higher than the feed intake of chickens in T1, T3, and T4. There was no significant difference in PCV percentage, but there is a significant (p < 0.05) difference in lowering total serum cholesterol from T1 to T4 but higher in HDL cholesterol. Layers using the blended use of spices with drinking water (T4) had lowered total cholesterol levels in the blood compared to other treatments.

Keywords: Blood Parameters, Extract, Layers Performance, Onion, Rosemary

1 Introduction

Natural medicinal products originating from herbs and spices are used as feed additives for poultry (1). These plant-derived products have proven to be natural, less toxic, and residue-free and are thought to be ideal feed additives in animal feed production (2). This has increased the pressure on the poultry industry to find adequate alternatives, such as medicinal plants that can be used instead of antibiotics in animal nutrition (3, 4). The medicinal plants and their bioactive components are presently important in animal and poultry production and health care systems because they promote growth and production, enhance immunity, and safeguard health (5).

Rosemary (*Rosmarinus officinalis*. *L*) and onion (*Allium cepa L*.) are among the herbal medicinal plants that exert a potential effect on animal feed industry development (6, 7). Phenol compounds in rosemary, such as diterpenes, carnosol, carnosic acid, methyl carnosic, rosmarinic acid, and caffeic acid, play a vital role in antioxidant and antimicrobial activity against bacterial growth. The anti-oxidative activity of rosemary is due to phenolic terpenes, rosmanol, and rosmarinic acid, which improve the oxidative stability of poultry meat (8).

The onion (*Allium cepa L.*) is well known for its effective prevention and treatment of diseases by antioxidant, antihypertensive, antithrombotic, antibiotic, and anticarcinogenic effects with its variable biochemical functions (9). It contains sulfur-containing compounds, which are sources of methionine, cysteine, and amino acids. It has effects in lowering the cholesterol level in blood plasma or serum and has a vital role in the growth of the birds (10). However, in Ethiopia, more research needs to be conducted to determine the combined effects of rosemary leaf and onion extract mixture on the productive performance of layers. Therefore, this study evaluated the effects of rosemary and onion extracts on layer performance, egg quality, and blood parameters.

2 Materials and Methods

Table	1.	Experimental	Lavout
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2.1 Description of Study Site

The study was conducted at Haramaya University poultry farm, 525 km from Addis Ababa. The site is situated at an altitude of 1980 millimeters above sea level, $9 \circ 26$ 'N latitude, and $42 \circ 3$ ' E longitude. The area has an average annual rainfall of 741.6 millimeters. The mean annual minimum and maximum temperatures are 8.25 °C and 23.4 °C, respectively (11).

2.2 Preparation of Experimental Feed Additives

The measured amount of rosemary (1.4 kg) and onion (4 kg) were purchased from the local market (Harar). The onion bulbs were cleaned, the peeled and the root was removed, then cut into small pieces and spread on a plastic sheet for two days to dry and make it easier for grinding following the method of (12) and finally grated by mixer and put in a plastic container at room temperature. The rosemary leaf was cleaned, separated from steam, and grated by a grinder to make powder following the methods of (13). Then, 20 g of powder was weighed from each spice and mixed with one litter of distilled water in a separate plastic container. The mixture was shaken thoroughly to get the diluted solution of the juice. The obtained solution was kept overnight for about 12 hours at room temperature and then filtered and poured into separate plastic containers. Then, each amount of extract was divided into experimental pens (replication) for daily use (14).

2.3 Experimental Design and Treatments

The chickens were randomly allocated to four treatments, each with three replicates. Each replication consists of 10 layers and one cock in a completely randomized design (CRD). The measured amount of onion and rosemary extract were allocated to treatments based on the experimental design as 4 ml of onion extract (T_2), 4 ml rosemary extract (T_3), and the combination of each 2 ml of onion and rosemary extract (T_4) before offering normal daily drinking water.

Treatments	Proportion of extracts in one litter of water	Replication	Cocks	Layers	Total
T_1	Control group	3	1	10	33
T_2	4 ml of onion extract in one litter of water	3	1	10	33
T ₃	4ml of rosemary extract in one litter of water	3	1	10	33
T_4	2 ml onion +2 ml of rosemary extract in one litter of water	3	1	10	33

2.4 Experimental Chickens and Management

A total of 132 White Leghorns (120 layers and 12 cocks), thirty-two weeks old, were taken from Haramaya University Poultry farm. The chickens were acclimatized to the experimental ration for 7 days and managed for 70 days. The experimental pens, watering, feeding troughs, and laying nests were carefully cleaned and disinfected. The experimental diet and water were provided in a group *ad libitum* throughout the experimental period.

2.5 Data collection and Statistical analysis

Feed consumed was determined as the difference between the feed offered and refusal for each replication. Feed intake was then calculated from feed consumed and divided by the total number of layers. All layers were individually weighed at the beginning and end of the experiment by sensitive balance, and then the average body weight was taken for each treatment. Body weight (B.W.) change was determined as the final and initial body weight difference. B.W. gain or loss was calculated as B.W. change divided by the number of experimental days (15). The feed conversion ratio was determined by calculating the weight of feed consumed and egg mass following the method of (16).

All eggs were weighed immediately after collection for each replicate, and the average egg weight was computed daily by dividing the total egg weight by the number of eggs for each treatment. The rate of lay for each treatment was expressed as the average percentage of hen-day egg production (HDEP %), and then egg mass was calculated by multiplying the average egg weight by (HDEP %) (17).

The hematological parameters (Hemoglobin, red blood cell, white blood cell counts, and packed cell volume) were given to a hematology analyzer, and about $20 \,\mu$ l of blood was mixed with diluent and lyzer to be analyzed by HUMACOUNT analyzer using an electrical impedance

procedure. Blood cells (WBC and RBC) were counted using an automatic blood analyzer as they produced resistance to electric signals when they passed through the solution (18). Micro hematocrit capillary tubes centrifuged the packed cell volume (PCV) at 3000 rpm for 5 minutes. The serum biochemistry indices (total cholesterol, HDL, LDL, total protein, albumin, and globulin) were determined by HUMALYZER using a spectrometry procedure (19). The serum was mixed with each reagent, incubated, and brought to the analyzer. The analyzer measures the analyses as the chemical reaction is produced and observance is detected. The difference between serum total protein and albumin determined the globulin value.

Finally, all collected data were analyzed using SAS software version 9.4. The significant differences between treatment means were located using the least significant difference (LSD) method. The following statistical model was used for data analysis.

$$Yijk = \mu + t_i + e_{jK}$$

Where:- y_{ijk} = the response variable

 μ = the overall population means,

 T_i = treatment effect (t = rosemary, onion, and their mixture effects as feed additives),

EjK = experimental error.

3 Results and Discussion

3.1 Chemical Composition of Layers Ration and Feed Additives

The nutritional composition of commercial layers' ration and experimental feed additives were analyzed for DM, CP, C.F., EEEE, and Ash at Haramaya University animal nutrition laboratory following the procedure of AOAC (2012). The following formula estimated their metabolizable energy: ME (Kcal/Kg DM) = 3951+54.4 EEEE - 88.7 CF- 40.8 Ash) (20).

Table 2. Dietary composition of commercial layers' ration

Ingredients	DM%	CP%	EE%	CF%	Ash%	ME (kcal/kg)
Maize	90.10	8.50	11.50	3.90	3.80	3595.5
Noug seed cake	93.50	29.40	12.00	11.50	10.20	3130
Wheat short	90.50	150	3.50	5.80	3.50	2955
Soybean meal	92.50	38.40	3.80	5.60	5.50	3410

DM=Dry Mater, CP=Crude Protein, EE=Ether Extract, CF= Crude Fiber, ME= Metabolizable Energy, Kcal= Kilo calorie, and Kg= Kilogram.



Spices	DM%	CP%	EE%	Ash%	CF%	ME (Kcal/Kg DM)
Onion	81.54	1.17	1.65	4.64	5.013	3406.79
Rosemary	91.74	9.68	5.95	8.16	25.03	7121.59
Layer ration	90.50	16.5	4.80	11.35	8.50	2936.45

Table 3. Chemical Composition of onion and rosemary and treatment ration

DM=Dry Mater, CP= Crude Protein, EE= Ether Extract, CF= Crude Fiber, ME= Metabolizable Energy, Kcal= Kilo calorie, and Kg= Kilogram.

3.2 Production Performance of White Leghorn Layers

Table 4. Effects of onion and rosemary extracts on laying performance of White Leghorn.

Parameters / Treatment	T_1	T_2	T_3	T_4	SEM	SL
Feed Intake (g/bird/day)	94.65 ^{ab}	97.59ª	94.14 ^b	93.39 ^b	0.72	**
Initial B.W. (g/bird)	1209.30	1178.30	1205.00	1198.30	12.23	NS
Final B.W. (g/bird)	1292.70	1271.70	1296.70	1280.00	11.11	NS
BW Change (g/bird)	83.40 ^{bc}	93.40ª	91.7 ^{ab}	81.70°	5.07	*
BW gain (g/bird)	1.30 ^{bc}	1.46 ^a	1.43 ^{ab}	1.27 ^c	0.04	*
Total egg/hen	41.83 ^{ab}	42.83ª	41.96 ^{ab}	41.50 ^b	0.41	*
HDEP (%)	50.63 ^{ab}	52.49ª	51.21 ^{ab}	49.21 ^b	0.65	*
Egg mass (g/hen/day)	29.79 ^b	35.44 ^a	28.63 ^b	21.00 ^c	0.43	*
FCR (g feed/g egg)	3.19 ^b	2.75 ^b	3.26 ^b	4.41 ^a	0.00	*

^{a,b and c} Means within a row with different superscripts are significantly different, *=Significant at (p< 0.05), **=Significant at (p=0.01), SL= Significant Level, SEM=Standard Error of Mean, g = gram, BW= Body Weight, HDEP = Hen Day Egg Production, FCR = Feed Conversion Ratio, EM= Egg Mass (g/hen/day), T₁ = Control Group (No additive), T₂ = 4 ml of onion extract added in one liter of water, T₃ = 4 ml of rosemary extract added in one liter of water, T₄ = 2 ml/ rosemary +2 ml onion extracts in one liter of water.

3.2.1 Feed intake

The effects of onion, rosemary, and their combination extract infusion offered in drinking water on production performances are shown in Table 3. During the entire experimental period, there was no record of experimental extract water left over, which means the birds used all amounts of the extract. The feed intake was significantly (p<0.01) highest in T₂ compared to T₃ and T₄. The result was agreed (21), who noted that including onion powder (p < 0.01) increased the feed consumption of layers. This was due to the bioactive S-compounds of onion, such as cysteine, methionine, and amino acids, which might enhance feed efficiency and nutrient utilization. The increased feed intake of layers using onion extract in drinking water was also reported by (22). However, T₁, T₃ and T₄ statistically similar. The lower feed intake in T₃ and T₄ might be due to the bitter test produced by rosemary extract, which reduces the feed consumption.

Onion extract can help maintain beneficial gut microorganisms and improve nutrient absorption. This corresponds with (23), who reported the effects of onion on poultry performance due to some of its compounds, such as phenols, polyphenols, terpenoids, polypeptides, lectin, alkalis, and essential oils that stimulate digestion and promote growth. It stimulates bile acid synthesis and pancreatic enzyme activity, mainly lipase and amylase, ultimately improving fat digestion (23). This is a quality of aromatic oils present in onion, which enhances digestion. The study of (10) reported that onion administration enhanced villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in birds' duodenum, jejunum, and ileum.

3.2.2 Body weight change and gain

The layer's initial and final body weights were statistically non-significant (p>0.05) among the treatments. This was due to the use of layers with similar initial body weight. In line with this finding, (24) also reported no differences in the final body weight of rosemary powder. This study revealed that the influence of spices inclusion in drinking water on body weight change and gain was significant (p<0.05).

 T_2 and T_3 recorded significantly higher body weight change and gain than T_1 and T_4 . This could be associated with efficient nutrient utilization because of the biological activities of phenolic compounds in onion and rosemary extracts individually. Congruently, (25) reported higher body weight for layers who drank water with onion extract.

In addition, using onion in diet can reduce blood glucose and lead to hypoglycemia, which stimulates the nervous system for higher feed intake, which can cause increased



weight gain (25). This is consistent with the findings of (25, 26), who reported positive effects on B.W. change of birds fed diets containing onion powder compared to the control group. The lower B.W. gain was recorded in T_4 . This might be due to the bitter test produced by the combination of the onion and rosemary extracts, resulting in decreased feed consumption.

3.2.3 Feed conversion ratio, egg production, and egg mass

Layers fed on onion blended with rosemary extract with drinking water (T₄) were recorded with the highest feed conversion ratio (FCR) than other treatments (Table 3). The lowest and the best FCR was observed in T₂. This is an indication of the better production performance of layers. Moreover, (10) noted that Allicin is the bioactive found in onion that improves and regenerates the physiological structure of the intestinal epithelium layer and enhances crypt depth and villus height, which ultimately supports its digestive capacity through increased absorption of nutrients and assimilation.

The additives may act in different mechanisms, affecting feed intake and conversion, stimulating the secretion of digestive enzymes and gastrointestinal motility, as well as immune and endocrine functions in addition to their antioxidant, antimicrobial, antiviral, anti-inflammatory, anthelminthic, and coccidiostats activities. These compounds increase feed efficiency by decreasing the harmful microbial population in the gut and improving health levels (27). The variation in feed conversion ratio in this study was mainly due to egg production increment as a result of onion and rosemary administration in drinking water, which is in line with the report of (28), who showed variation in feed conversion efficiency because it is highly dependent on the number of eggs produced, feed consumption and egg weight in layer hens. The feed conversion ratio increased with adding onion extract for laying hens, significantly improving the growth performance and egg quality of laying hens.

Total egg number (EN) and egg mass (EM) were significant (p < 0.05). According to the data presented in Table 3, no significant difference was observed between T_3 and T_1 regarding egg number (EN) and egg mass (EM). In addition, the egg number (EN) between T_3 and T_4 was not statistically significant. This is consistent with the report of (20), who noted the effects of polyhedral medicinal plants on layers of egg production performance (Table 3). The Highest Hen-Day Egg Production (HDEP) was recorded in T₂, whereas the lowest was in T₄. The blended use of the two spice extracts has resulted in lower HDEP than the separate use. The HDEP of T₃ and T₄ sharply increased during the first two weeks but slowly increased up to the fifth week, and then, after, it slowly declined (Figure 1). However, the figure showed that layers fed on T₂ diets recorded higher HDEP throughout the study period starting from the second week.

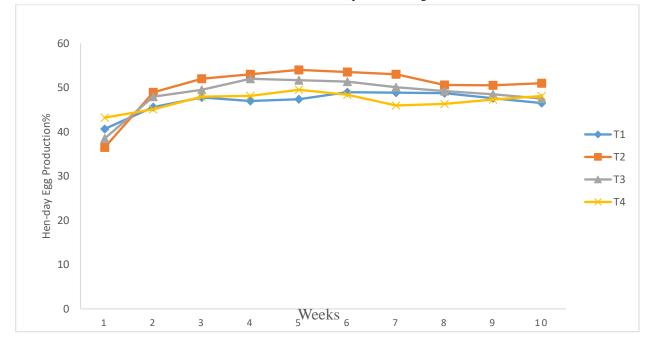


Figure 1. Weekly HDEP% of White Leghorn offered onion and rosemary extract in drinking water.

The improved HDEP was obtained due to the biological activities of phenolic compounds found in onion and

rosemary, which cause feed utilization improvement or feed efficiency. The result of (29) was that HDEP was improved



by adding rosemary to the diet layers. The improvement in egg production with phytogenic additives may be due to the provision of certain compounds that improve digestion and absorption of nutrients in the digestive tract. The extracts of medicinal plants increased the secretion of digestive enzymes, enhanced nutrient digestibility, and improved the egg production performance of layers. This finding indicated that including onion and rosemary extract in the drinking water of layers could give the best overall production performance of layers. The result was also agreed with (6), who noticed a significant (p<0.05) increase in egg production and egg mass.

3.3 Hematological and Serum Biochemical Analysis of White Leghorn Layers

3.3.1 Serum Biochemistry Analysis

 T_1 Parameters / Treatment T_2 T_3 T_4 SEM SL Serum biochemistry 164.80^a 159.98^{ab} 160.50^{ab} 158.98^b 1.07 * Total cholesterol (mg/dl) * HDL-C(mg/dl) 94.87^{ab} 93.28^{ab} 90.82^b 98.22^a 1.76 * LDL-C (mg/dl) 46.43^{ab} 43.42^{ab} 41.97^b 48.75^a 1.75 * TP (g/dl) 4.13^b 4.56^a 4.50^a 4.16^{ab} 0.06 Albumin (g/dl) 2.90 2.93 2.85 0.09 NS 2.73 Globulin (g/dl) 1.40^{b} 1.60^a 1.31^b 0.06 1.67^a * Hematology 2.34^b ** RBC (10⁶/µl) 2.65ª 2.57^a 2.51^{ab} 0.07 WBC (10⁴/µl) 1.85^a 1.65^{ab} 1.87^a 1.45^b 0.16 * * Hb (g/dl) 10.22^b 10.62^a 10.28^b 10.16^b 1.69 PCV (%) 33.17 33.17 33.00 31.67 0.66 NS

Table 5. Effects of onion and rosemary extracts on hematological and serum biochemistry of White Leghorn.

^{a-b}=Means with in a row with different superscripts are significantly different; HDL-C= High Density Lipoprotein cholesterol, LDL-C= Low Density Lipoprotein Cholesterol; *=Significant at (p<0.05); **= Significant at (p<0.01); NS= Not-significant. RBC=Red Blood Cells, Hb= Hemoglobin, PCV=Packed Cells Volume, WBC=White Blood Cells, TP=Total Protein.

Onion, rosemary, and their mixture extracts offered in drinking water significantly affected the laying hens' serum total cholesterol, HDL cholesterol, LDL cholesterol, and total protein (T.P.) (Table 4). According to the study of (30), the effects of onion have been qualified to its sulfur-containing principles, which oxidize thiol compounds either present free or combined with a protein necessary for lipid synthesis.

The inclusion of onion, rosemary, and the combined extracts in drinking water as feed additives was found to cause a decreased (p>0.05) values of total cholesterol level, and the lowest value was no significant difference between T₄ with T₂ and T₃, which might be of the synergetic effect of the combination of onion and rosemary extracts. HDL-cholesterol and LDL-cholesterol concentrations were shown to have a significantly lower value in T₃. The reduced total cholesterol and LDL content may reflect the hypocholesterolemia properties attributed to the defatted part of the leaves, which are rich in fibrous content and may block intestinal cholesterol absorption. The study (31) revealed that rosemary extract caused a numerical decrease in serum cholesterol, LDL-cholesterol, and lipid concentration levels and increased triglyceride levels. Moreover, (29) concluded that rosemary

extract numerically decreases serum cholesterol levels of triglycerides and HDL and LDL cholesterol.

The study (32) reported that dietary onion effectively lowers serum cholesterol levels. According to (16) who reported that LDL-cholesterol, HDL-cholesterol, and triglyceride were not significantly affected by the diet containing rosemary oil. (25). Reported that using onion bulbs in the broiler diet decreased triglyceride and total cholesterol in blood serum. As (6) stated, the diet enriched in rosemary numerically reduced serum triglycerides and total cholesterol, besides LDL-cholesterol concentrations (33). They concluded that adding rosemary powder to chicken diets decreased serum concentrations of triglycerides, total cholesterol, and LDL. The findings of (25) concluded that rosemary oil might produce hypoglycemic activity through independent insulin secretion, inhibiting endogenous glucose production or inhibiting intestinal glucose absorption, which reduces LDL cholesterol accumulation.

Similar results were concluded by (34), who stated that blood triglyceride and total LDL-cholesterol concentrations were significantly reduced by adding rosemary as feed additives to chicken diets, but HDL-cholesterol increased. Moreover, the layers-fed diets infused in drinking water with



rosemary leaves powder at 0.5% and 1% levels had lower total lipids. The findings of (33) stated that the values of HDL and total cholesterol concentration decreased significantly (p<0.05), with laying hens receiving a ratio of 0.5% and 1% onion powder.

There was a significant (p<0.05) difference in total blood protein concentration, in which the highest value was recorded in T₂ and T₃. The serum albumin content showed no statistical (p<0.05) difference.

3.3.2 Blood Hematological Analysis

There were significant (p < 0.05) differences in RBC, WBC, and Hb concentration, but the PCV percentage was not significant (p < 0.05). The probable reason for the significant result could be the effects of onion and rosemary bioactive compounds on hematological and serum biochemical indices. The bioactive sulfur organic compounds, including S-Methyl cysteine sulfoxide and sally cysteine sulfoxide found in onion, can affect the concentration of blood lipids, protein, and glucose. These results were consistent with the findings of (25), who noticed that the onion extracts affect lipid metabolism through fatty acid transportation and can increase lipid utilization. The findings of (27) noticed that alliums enhanced the relative weight of the spleen and thymus through increased garlic and onion supplementation, which was recognized to increase lymphocyte proliferation and WBC counts. The lowest results of RBC and WBC are shown in T₄. This contradicts the findings of (6), which reported that medicinal plants and their components could activate immune functions such as lymphocyte proliferation, phagocytosis, RBC, and Hb and WBC.

The Hb was significantly highest in T₂. This could be due to the phenolic compounds found in onion and rosemary as good sources of iron, which were the components of Hb and determine the oxygen-carrying capacity of red blood cells. This result aligns with the findings of (28), who reported that dieting with rosemary leaves significantly increased Hb, RBC, WBC, lymphocytes, and monocytes compared to the control group. Similarly, (33) reported that onion supplementation in poultry diets was used as an iron source, an essential component of Hb, and to carry and transport oxygen in RBCs.

4 Conclusion

The current study generally concludes that the inclusion of 4 ml of onion extract in drinking water is recommendable based on the overall performance of the White Leghorn layer. On the other hand, rosemary may be used to improve egg production and egg mass, lowering total and LDL cholesterol. Indeed, further detailed research is needed to assess the identification of active chemical compounds in onion and rosemary and their effect at higher proportions of extracts in drinking water on the performance of layers.

Acknowledgements

None.

Conflict of Interest

The authors argued and declared that there were no competing interests.

Author Contributions

Seyoum Bekele, the first author, generated the primary data, which was manipulated, organized, analyzed by the SAS computer, and interpreted. Meseret Girma (PhD, Associate Professor) designed the manuscript for publication format, translated it, and corrected the English language grammar. Ewunetu Kebede (Assistant Professor) also designed the manuscript for publication format, translated it, and corrected the English language grammar.

Data Availability Statement

The data produced and examined during this study are not openly accessible but can be obtained from the corresponding author upon a reasonable request.

Ethical Considerations

The article was prepared on the topic: *Effects of Rosemary* (*Rosmarinus officinalis L.*), Onion (Allium cepa L.) Extracts and their mixtures as Feed Additives on Layers Performance and some Blood profiles of White Leghorn at Haramaya University Poultry Farm Research Center, Ethiopia, followed the international guiding principle for Biomedical Research Involving Animals listed under Article 2012 of the International Council for Laboratory Animal Science (ICLAS). Therefore, the School of Animal and Range Sciences Animal Ethics Committee and Committee for Control and Supervision of Experiments on Animals in Ethiopia approved the experimental procedure dated 5 May 2021.



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