RESEARCH ARTICLE

Haematological indices of broiler chickens fed fortified-fermented yam peel as replacement for energy source

Muhammad Haruna Garba[1*], Folashade Gift Ntagbu[2], Bulus Lekene Jatau[3], Sa'adu Adamu abdullahi[4], Nasir Salihu[1], Babatunde Kehinde Oluwafemi[4]

[1]Department of Biochemistry, Federal University, Dutse, Jigawa State, Nigeria
[2]Department of Animal Production Technology, Microbiology and Nutrition Unit, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Niger State, Nigeria.
[3]Poultry and Monogastric Unit, Department of Animal Health and Production Technology, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Niger State, Nigeria.
[4]Department of Basic Sciences, Federal College of Wildlife Management, P.M.B. 268, New Bussa, Niger State, Nigeria.

***Correspondence:**
Dr. Muhammad Haruna Garba
Email: mharunagarba@gmail.com

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ABSTRACT

Background: Livestock represents an important source of high-quality animal proteins. An inadequate supply of feeds and forages for optimum production is the major constraint to global livestock production. To avert the imminent protein malnutrition, the problem of animal protein scarcity must be addressed. In the present study, a five weeks' study was conducted using 75 unsexed Anak broilers to determine the effect of fermented yam peels meal (FYPM) as a partial replacement for maize on the performance and haematological indices of finishing broilers. **Methods:** Seventy-five grower broiler chickens were randomly allotted to five (5) groups (T1, T2, T3, T4, and T5). T1 is the control. The birds were fed diets containing 0, 10, 15, 20, and 25% fermented yam peel meal as a replacement for energy source (Maize). Each group was replicated three times with 5 birds per replicate in a completely randomized design (CRD). Feed and water were provided ad libitum for the period of five weeks. **Results:** Results showed that significant differences ($p \leq 0.05$) exist among the treatment with respect to body weight gain, feed intake, and feed conversion ratio. Mortality was recorded over the period of the trial, particularly in the group with a higher inclusion level. The experimental diets had a significant ($p \leq 0.05$) effect on haematological indices such as Red blood cell (RBC), packed cell volume (PVC), haemoglobin (Hb), mean corpuscular Concentration (MCHC), white blood cell (WBC), and its differentials. There was also a significant increase ($p \leq 0.05$) in the platelet counts (PLC) with the corresponding increase in the fermented yam peel. **Conclusion:** The results of this study revealed that 20% fermented yam peel meal can replace maize in the diet of finishing broilers with better performance.

Keywords: Yam peel; fortification; fermentation; performance; biochemical indices

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1.0 Introduction

Livestock represents an important source of high-quality animal protein, providing about 36.5 per cent of the total protein intake of Nigerians. It is one of the highest investments in agriculture with a net worth of N250 billion [1].

Feed is the largest and most important component to ensuring safe, abundant, and affordable animal proteins and animal feeds play a leading role in the global food industry [1]. An inadequate supply of feeds and forages for optimum production is the major constraint to global livestock production. At the same time, due to rapid urbanization and industrialization, land devoted to forage production does not likely to increase soon [3]. The Food and Agriculture Organization of the United Nation projected that the world's population is expected to reach more than 9 billion by 2050 and there must be a 60% increase in food production to keep phase with the trend. More so, with the advances made in animal production technologies, it is believed that animal protein production will grow, even more, meats (poultry/swine/beef) will double, as well as dairy, and fish production will almost triple by 2050 [4].

To avert the imminent protein malnutrition, the problem of animal protein scarcity in Nigeria and other developing nations that have attained a deplorable status will have to be urgently addressed [5]. The high cost of conventional ingredients for feed making which has made monogastric animal feed a major cost in production has been blamed as the cause of this problem [6]. Feeds and feed

resources had been rated by Dafwang [7], as constituting 70% of the total cost of animal production in Nigeria. Escalation in the prices of conventional feed ingredients especially the energy sources such as maize, sorghum, etc. has been attributed to this ugly situation [8]. Efforts to reverse the trend brought about the quest to search for alternative feedstuffs.

Conversion of lignocellulose materials such as yam peels into value-added products which have the potential to provide solutions to problems of animal nutrition such as inadequate intake of protein and calories relies on fermentation which is an essential technology employed. This process will also reduce waste pollution as well as increase the quality of animal by-products [9]. Rumen microbes are widely accepted due to the long history of their use in traditional fermentation and their relative safety compared to some soil micro-organisms [10, 11].

Yam (*Dioscorea spp*.) is an important food crop in Africa. Nigeria is the leading producer of yams in the world accounting for over 65% (with 38 million metric tonnes) of the world production in 2012 [12]. Other regions where yam is produced include the Americas, Caribbean, South Pacific, and Asia. The Food and Agriculture of the United Nations FAO, [13]. reported that white yam (*Dioscorea rotundata*) is the most important cultivated yam. It is processed into a wide range of products in different parts of Africa and other regions of the world. In tropical Africa, yam flour (pounded-yam), yam gruel (mala), pounded yam (iyan), fried yam (dundu), fried grated-spiced yam (ojojo), and other products are made from yam resulting in large amounts of yam peels which is very low in protein, consequently, it is either discarded or burnt resulting in pollution of air, land, and water.

This research is therefore set to investigate the changes that may occur in the haematological parameters of broiler chickens fed a diet containing a graded level of fermented yam peel as a substitute for energy sources (maize).

2.0 Materials and methods

2.1 Experimental site

The research was conducted at the teaching and research farm of the federal college of wildlife Management, New- Bussa Niger State. New-Bussa The experimental station (New Bussa) sits at 9o53'N,9.883oN, and 4o31'E, 4.517oE [14]. The research work was carried out between May to July (the early part of the rainy season)

2.2 Source of the yam peel

About 50 Kg of dried yam peel was collected from volunteer donors in Kpakungu (Minna) and Wawa village (New Bussa) all in Niger State. It was then washed trice to eliminate the dust and minimise fungal contamination as much as possible. It was dried at room temperature and constantly monitored until the water activity (aw) was found to be 0.70. It was then packaged in a clean polythene bag until required for use.

2.3 Source of rumen microbes

Rumen contents were obtained from the freshly slaughtered animals (Cows in particular) at the New Bussa abattoir. The rumen content was diluted with secured distilled water in the ratio of 1: 5 v/v. It was vigorously shaken then filtered through a sieve of pore size 0.5mm. The filtrate was placed into an appropriate container and securely tied and stored in the refrigerator at 4oC until required for use.

2.4 Fermentation of the Sample

The sample material (Yam peel) was pulverized using a hammer miller into smaller sizes that can pass through a sieve of pore size of 1.0mm. A five-kilogram weight (5Kg) was then placed into a plastic paint container of 25 L capacity. It was then made into a paste by adding a sufficient quantity of water. To the paste was then added 1 L of the rumen filtrate and thoroughly mixed by stirring with a clean and sterilized stick. More water was further added until it completely becomes submerged and fortification of the fermentation process with 10g urea granules was done. Thereafter, a polythene sheet measuring one-meter square (1.0 M2) was used to tightly cover the mouth of the container. It was kept and allowed to ferment anaerobically for seven days.

2.5 Experimental birds

Seventy-five indigenously developed varieties of broilers were procured from the NEFRADEY farms in Ilorin, Nigeria. The birds were divided into 5 (A – E) treatments in a completely randomized design, with 3 replicates per treatment and 5 birds in each replicate.

Twenty-one pens were made and the replicates were randomly allotted using the lottery method under a deep litter system and reared for 6 weeks.

2.6 Broilers and housing

Broiler chickens were obtained at 1 day of age; they were vaccinated for Marek's disease at the hatchery and Newcastle disease and infectious bronchitis at the test site on the study start day. The broilers were randomly assigned to concrete-floored pens (1.5 m × 0.9 m, providing approximately 0.09 m2 per bird), with approximately 13 cm of clean wood shavings in an environmentally controlled building with incandescent lighting. Incandescent lighting was provided for 23 to 24 h/d for approximately the first 4 days of the study and 10 to 16 h for the remainder of the study. The target room temperature was 34°C at the start of the study and was gradually decreased each day to a target room temperature of 23°C from day 30 through the remainder of the study. Water and feed were available for ad libitum consumption throughout the experiment.

2.7 Diets

Diets were formulated to meet or exceed NRC [15]. values for broiler chickens. A coccidiostat, salinomycin (Sacox, Intervet Inc., Millsboro, DE), was included in all diets at a level of 50 g/ton. Broilers were fed a starter diet from day 0 to 28. For the rest of the study (days 28 to 63), broilers were fed a grower-finisher diet.

2.8 Experimental design

Seventy-five (75), day-old Anak broiler chicks were allotted into five groups (A-E) in a completely randomized design. Each group consists of fifteen (15) chicks assigned into three replicates with each replicate consisting of five chickens**.**

Treatment 1- was the standard control substituted with 0% fermented yam peel as a replacement of energy source at the finisher stage

Treatment 2- was the standard control substituted with 10% fermented yam peel as a replacement of energy source at the finisher stage

Treatment3- was the standard control substituted with 15% fermented yam peel as a replacement of energy source at the finisher stage

Treatment 4- was the standard control substituted with 20% fermented yam peel as a replacement of energy source at the finisher stage

Treatment 5- was the standard control substituted with 25% fermented yam peel as a replacement of energy source at the finisher stage

2.9 Bodyweight measurements

Individual body weights were recorded at the beginning of the experiment and further bodyweight increments were recorded at the end of each week to monitor the pattern of body weight changes. Feed and water were supplied ad-libitum for about 3 weeks before the commencement of the experiment.

2.10 Feed consumption

The daily amount of the diet was weighed and offered to the birds in each group. The feed consumption in each replicate was recorded weekly by subtracting the weight of residual feed from the total quantity of feed supplied during the respective week.

2.11 Feed conversion ratio

The feed conversion ratio (FCR) was determined through the relationship between the amount of feed consumed (FC) to the body weight gain (BWG) under each group of birds

$$\textbf{FCR} = \frac{FC(g)}{BWG(g)}$$

2.12 Livability

Mortality in the respective group was recorded at occurrence in the starter and finisher periods.

2.14 Hematological parameters

Haematological parameters including red blood count (RBC), haemoglobin (Hb) concentration, packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (DLC) were evaluated as per the procedures outlined by Kone *et al* [16].

2.15 Statistical analysis

Data on various parameters obtained during the experiment was analyzed as Completely Randomized Block Design according to the

SAS., 2013 [17]. SAS user's guide statistics. Software Version 9.4.SAS Inst Institute, Inc., Cary, NC, USA.

3.0 Result

3.1 Proximate composition of the fermented and unfermented yam peel

Subjecting the yam peel to fermentation process brought about a decrease in the moisture and fat content; increase in the concentration of crude protein; ash contents and crude fiber with no significant changes in the nitrogen free extract as revealed in Table 1. The composition of the experimental diet is such that, it ensures the presence of all the nutrients required for the fast and healthy growth of the experimental birds (Table 2).

Table 1: Proximate composition of the fermented and unfermented yam peel

Proximate	FYM (%)	UFYM(%)
Moisture	3.19 ± 0.23	10.56 ± 0.13
Ash	3.80 ± 0.26	1.581 ± 0.03
Crude protein	6.81 ± 0.59	4.60 ± 0.35
Crude fiber	2.98 ± 0.7	1.513 ± 0.012
Crude fat	3.04 ± 0.13	0.432 ± 0.09
Nitrogen free extract	80.35 ± 0.9	80.664 ± 0.20

Values are the mean of triplicate measurements ± Standard deviation (SD). FYM = Fermented Yam Peel, UFYM = Unfermented Yam Peel

Table 2: Percentage of composition of experimental diet at finisher phase

Ingredient	T10% (Kg)	T2 10 % (Kg)	T3 15% (Kg)	T4 20% (Kg)	T5 25% (Kg)
Maize	50.2	45.18	42.67	40,16	37.65
Fish Meal	10	10	10	10	10
GNC	6.0	6.0	6.0	6.0	6.0
Soya Beans	20	20	20	20	20
Wheat Offal	9.7	9.7	9.7	9.7	9.7
Bone Meal	3	3	3	3	3
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Vitamins Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0,25
Powder coccidiostart	0.05	0.05	0.05	0.05	0.05
Growth Enhancer	0.05	0.05	0.05	0.05	0.05
FYP	0.00	5.02	7.53	10.04	12.55
Total	**100**	**100**	**100**	**100**	**100**

FYP = Fermented Yam Peel

3.2 Performance of broiler finisher chicken fed fortified-fermented yam peel

The best performance in all the treatments occurred in T4 with 20% inclusion while the least performance with a high FCR value manifests in T2 at 5% inclusion. Treatments 3 and 5 compares favorably with the standard control T1 (Table 3).

Table 3: Performance of broiler finisher chicken fed fortified-fermented yam peel

Parameters	T1(0%)	T2(5%)	T3(10%)	T4(20%)	T5(25%)	SEM
Mean initial body weight(g)	335.59^{a}	275.77^{d}	325.78^{b}	304.91^{c}	303.55^{c}	8.51
Mean final body weight(g)	1367.22^{c}	949.50^{d}	1434.08^{b}	1530.13^{a}	1370.83^{c}	57.78
Mean body weight gain(g)	1036.63^{b}	673.80^{a}	1108.30^{c}	1225.22^{d}	1067.28^{a}	43.27
Mean feed consumed (g)	1440.9^{c}	950.10^{a}	1440.80^{c}	1531.53^{d}	1387.46^{b}	57.78
Feed conversion ratio (FCR)	1.39	1.41	1.30	1.25	1.30	

Values on the same row with different superscripts are significantly different ($p \leq 0.05$)

3.3 Haematological indices of broiler chickens fed fortified-fermented yam peel

There seems to be a negative correlation between the red blood cells count (RBC) and the concentration of the experimental diet consumed, with T1(0%) having the highest value and T5 (25%) having the least value. While the packed cell volume (PCV) appeared to be lowest in T2 and T1 and highest in T5, a similar trend occurred in the case of haemoglobin concentration (HB). The Platelet count (PLC) is highest in T4, T2 and lowest in T3. Incidentally, the total white blood count (TWBC) is lowest in T1 and highest in T4 as revealed in Table 4.

Table 4: Haematological indices of grower broiler chickens fed ammonium-fortified fermented yam peel diet as a replacement for an energy source

	1	**2**	**3**	**4**	**5**	**SEM**
RBC	9.70^{e}	7.22^{d}	5.72^{c}	4.37^{b}	3.15^{a}	0.63
HB	4.30^{a}	8.60^{ab}	4.55^{a}	10.75^{b}	11.30^{b}	1.01
PCV	13.00^{a}	25.50^{b}	10.30^{a}	32.00^{C}	33.50^{C}	3.27
MCV	104.00^{b}	87.50^{a}	96.00^{ab}	116.00^{c}	99.00^{b}	2.79
MCH	29.00^{a}	36.50^{ab}	35.50^{ab}	51.00^{c}	41.00^{bc}	2.35
MCHC	51.50^{b}	36.00^{a}	32.00^{a}	33.50^{a}	35.50^{a}	3.66
PLC	270.50^{a}	618.00^{b}	244.00^{a}	814.50^{c}	570.50^{b}	61.48
TWBC	10.75^{a}	22.65^{ab}	15.65^{a}	43.70^{a}	19.30^{a}	4.12
NEU	9.00^{a}	11.00^{ab}	17.00^{b}	14.50^{ab}	16.50^{b}	1.14
LYM	86.00^{b}	84.00^{b}	72.50^{a}	83.00^{b}	75.00^{a}	1.71
MON	6.00^{ab}	5.00^{ab}	10.50^{b}	2.50^{a}	8.50^{ab}	1.02
EOS	6.00^{ab}	5.00^{ab}	10.50^{b}	2.50^{a}	8.50^{ab}	1.02
BAS	6.00^{ab}	5.00^{ab}	10.50^{b}	2.50^{a}	8.50^{ab}	1.02

Values on the same row with different superscripts are significantly different $p \leq 0.05$. RBC = Red blood cell count, Hgb = Haemoglobin, PCV = Packed cell volume, MCV = Mean cell volume, MHC = Mean haemoglobin concentration, MCHC = Mean cell haemoglobin concentration, PLC= Platelet count, TWBC = Total white blood count, Neu = Neutrophils, Lymp = Lymphocytes, Mon = Monocytes, Eos = Eosinophils, Bas = Basophils.

4.0 Discussion

As indicated in Table 1, there has been a decrease in the moisture content but a significant increase in the crude protein, fibre, crude fat and ash contents. These increases are of great importance as it reflects an increase in the nutritional quality of the sample material. However, as fermentation usually harbours both harmful and beneficial fungal organisms, such effects are revealed in the haematological indices of the sample organisms.

The activities of microorganisms in the course of fermentation of organic materials bring about changes both in nutrient composition, palatability and physicochemical properties. This, in most cases, brings about an increase in the nutrient composition of the products such as the protein that may emanate from the accumulated microbial protein. However, some bacterial and fungal toxins that may hamper or grossly retard the overall performance of the animal may be incorporated into the product obtained from fermentation [18].

A high concentration of protein and vitamins (about 200% higher than in the whole plant) has been obtained in a work reported by Kazimierz [19]. It was also reported that fermentation of alfalfa leaf fraction resulted in an increase in the protein content from 27 to 32% and decrease in fibre content by 12–13%; and also an increase in the value of carotene to the range of 120–150 mg. Such reports on the positive impact of fermentation on the nutritional content of agricultural by-products are corroborated by our findings in Table 1.

As reflected in Table 3, there is initial rejection, then followed by a gradual increase in the amount of feed consumed as the week's progresses, this could be due to initial rejection and subsequent acclimatisation to the taste and aroma of the included diet [1]. As a natural principle, the tendency of animals to gain an increase in body size is dependent not solely, on the quantity of the feed supplied, but partly due to the quality of the nutrients it contains. What qualifies feed as standard rather than just the quantity is its ability to provide nutrients that support body immunity, enhance growth, guarantee the repair of the worn-out tissues, support the strength and development of the body's skeletal and nervous systems and suppress the spread and proliferation of microbial pathogens [5]. The growth pattern shown in Table 4 seems to be in tune with the mentioned attributes of a good feed/diet.

On the overall note, it suffices to state that, considering the feed conversion ratios values across all the treatments (control inclusive), the performance of the experimental birds have not been encouraging and raise the question on the genetic purity of the breed used, the effect of the environment in which the trial was performed or a load of harmful microbial organisms contaminating the feed [21].

The red blood cells (RBCs) indices provide information about the size and quality of the red blood cells. This can be used to diagnose the cause and severity of anaemia and provide vital clues about other health conditions an animal may have [22]. From table 4, it could be observed that the RBC decreases as the inclusion of the FYP increases. When these raised values are compared to the normal ranges, it could be asserted that the raised values in T1 and T2 may be due to erythrocytosis or polycythemia vera caused by either dehydration or obstructive pulmonary diseases that truly manifest in the experimental birds.

The RBC indices are comprised of four different components known as the mean corpuscular haemoglobin concentration (MCHC), the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), and the red cell distribution width (RDW) [23]. Haemoglobin is the iron-carrying protein in red blood cells whose function is to carry oxygen. It is also the element that gives red blood cells their colour. Any alternation in concentration can cause the cells to appear more or less red. A Survey of Table 4 revealed that only T3 compared with the normal (T1) while other treatments showed higher values.

The mean corpuscular haemoglobin concentration (MCHC) is the average concentration of haemoglobin in the red blood cell. The MCHC tells whether an organism's red blood cells have more or less haemoglobin than what would be expected. Any value outside of the reference range is defined as follows: hyperchromic or hyperchromic depending on whether it is high or low[24],

conditions caused by either Autoimmune hemolytic anaemia, a condition in which the body's the immune system attacks its red blood cells or iron deficiency anaemia. A Survey of Table 4 revealed that there is no significant difference ($p \leq 0.05$) between the values for MCHC across treatments 2,3,4, and 5 while treatment 1 (control diet) appeared to be significantly ($p \leq 0.05$) higher

Mean corpuscular volume (MCV) measures the average red blood cell volume, meaning the actual size of the cells themselves. A normal range for MCV is between 80 and 96 femtoliters per cell [25]. Table 6 revealed that T2, T3 and T5 were within the normal ranges while T1 and T4 have their values above the normal ranges, which according to Simbaqueba *et al* [22]. is a sign of vitamin B12 and folate deficiency (both vitamin B12 deficiency and folate deficiencies are also called megaloblastic anaemia, due to the macrocytic RBCs.

Mean corpuscular haemoglobin (MCH) is the average amount of haemoglobin per red blood cell in a sample of blood. A normal range for MCH is between 27 and 32 picograms per cell [26]. The MCH value directly parallels the MCV value, and some physicians find that the test is redundant. As such, if the size of the red blood cells is large (as measured by the MCV), the amount of haemoglobin per red blood cell will be high (as measured by the MCH), and vice versa [27]. While the MCH can be used alone to determine if the anaemia is hyper-, hypo-, or normocytic, the MCV has to be considered along with the MCH since the cell volume directly affects the content of haemoglobin per cell [28, 29]. Table 4 showed that, aside from T1 (control), all the remaining treatments were above the normal range which could be a sign of insignificant concentration of haemoglobin per cell which may lead to hypoxia.

In a normal situation, a healthy animal has between 4,500 and 11,000 white blood cells per cubic millimetre of blood. Fluctuations in white cell numbers occur during the day; lower values are obtained during rest and higher values during exercise. An abnormal increase in white cell number is known as leukocytosis, whereas an abnormal decrease in number is known as leukopenia. White cell count may increase in response to intense physical exertion such as pain, certain disease states, such as infections and intoxications. The count may decrease in response to certain types of infections or drugs or associated with certain conditions, such as chronic anaemia, malnutrition, or anaphylaxis [30, 31].

White cells are grouped into three major classes such as lymphocytes, granulocytes, and monocytes each of which carries out somewhat different functions. Lymphocytes (B and T) are also very important in the immune system, with T cells being responsible for directly killing many foreign invaders [32]. B-lymphocytes (B cells), in contrast to the other types of white blood cells, are responsible for humoral immunity (in contrast to the non-specific immunity of other white blood cells) [33]. Neutrophils make up roughly half of the white blood cell population. They are usually the first cells of the immune system to respond to an invader such as a bacteria or a virus. Once released from the bone marrow these cells live for only around eight hours, but around 100 billion of these cells are produced by the body every day [34]. Eosinophils also play a role in fighting off bacteria and are very important in responding to infections with parasites (such as worms). These cells account for no more than 5% of the white blood cells in the bloodstream but are present in high concentrations in the digestive tract [30].

Basophils, accounting for only around 1% of white blood cells are important in mounting a non-specific immune response to pathogens [33]. Monocytes are the garbage trucks of the immune system. Around 5% to 12% of white blood cells in your bloodstream are monocytes, but their most important function is to migrate into tissues and clean up dead cells (among other functions) [35].

Taking into cognizance the afore-mentioned function of the white bold differentials, and the values obtained in Table 6, it could be observed that the values obtained were within the range excluding the lymphocytes that have values across all the treatments much above the normal range. This observation can be justified due to the fact that Lymphocytes fights foreign invaders and fermented yam peel is bound to harbour such organisms.

5.0 Conclusion

The substitution of Maize as an energy source with fermented yam peel (FYM), though, has minimised the cost of production, it can, however, be stated that the performance of

the grower broiler chickens has not been encouraging. The feed conversion ratio (FCR) determined showed the absence of some essential nutrients required for rapid growth in the fermented yam peel. More so, from the haematological analysis carried out, there is a loss of essential minerals and vitamins in the substituted diet. The result from the WBC and differentials also point to the fact that the fermentation has introduced some mycotoxins and possibly some un-attenuated microbial organisms. Summing these observations together, as promising and economical as this substitution appears to be, an improvement in the fermentation process needs to be employed to maintain/improve the nutrient content of the sample diet and also reduce the microbial load

Authors' contributions: This work was carried out as a collaboration between all authors. Authors MHG, NFG and BLJ designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors NS, SAA and BKO managed the analyses of the study, performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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Ethical approval: Authors hereby declare that "principles of Laboratory animal care" (NIH publication no. 85- 23, revised 1985) were followed, as well as Specific national laws where applicable. All Experiments have been examined and approved by the appropriate ethics committee.

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