Selection on Litter Size at Birth and Correlated Responses in Pre-weaning Litter Traits of Heterogeneous Rabbits in Southern Nigeria.

Abstract.

Mass selection to improve litter size at birth in rabbits will contribute to bridging the protein deficiency gap and improve income for rabbit farmers. The study investigated the effect of mass selection on litter size at birth and the correlated responses in pre-weaning litter traits in mongrel rabbits. One hundred and five female rabbits form a total of one hundred and ninety six (196) progeny (91 males and 105 females) generated from a mating scheme involving eighteen (18) bucks and forty eight (48) dams constituted experimental animals for the study. The selection criterion was litter size at birth (LSB). Animals were housed in three-tier hutches and fed with concentrate diet containing 15.81% protein, 2480 kcal energy and 8.22% fiber in the morning and green forages in the evening. Mass selection was performed on the criterion within three generations (F_S, F₁ and F₂). The experiment employed a nested design where dams were nested within sires. Pre-weaning litter traits studied with the criterion of selection were litter birth weight (LBW), kits birth weight (KBW) and number of kits born alive (NBA). Results revealed that LSB did not significantly (p>0.05) evolve with selection, although numerical improvements were observed. Sire and dam effects were not significant for the criterion. Values noted for LSB were 4.46±0.14, 4.48±0.15, 4.50±0.11 and 4.51±0.18 kits in F_S, F₁, F₂ and F₃ respectively. Realized selection responses per generation were 0.02, 0.02 and 0.01 in F_S, F₁ and F₂ respectively. Responses were less than expected in F₂ and F₃ generations of selection but similar in F_1 . The correlated changes in pre-weaning traits were non-significant (p>0.05) but with numerical improvements of 193.82±2.03, 193.38±2.27, 203.79±3.88 and 203.81±3.88 g for LBW and 4.09±0.16, 4.11±0.13, 4.20±0.09 and 4.21±0.17 for NBA but reduction of 43.56±1.16, 43.36±14, 42.99±0.65 g for KBW in the three generations respectively. It is concluded from this study that mass selection on litter size at birth (LSB) neither significantly improved the trait nor the pre-weaning traits that were correlated with it. Litter size at birth in heterogeneous rabbits could be genetically improved using other improvement strategies such as cross breeding.

Key words: Selection, Heterogeneous, Rabbits, Litter.

Introduction.

Worldwide population explosion and increasing incomes have created the need for increased food production and especially those of animal protein sources to meet the expected increase in demand (OECD-FAO, 2014). Animal genetic improvement is vital in this regard. Moreso, the genetic improvement of highly prolific, short gestation and nutritionally balanced meat producing animals like rabbits. Domestic rabbits have helped improved the livelihoods of resource poor households and contributed to poverty reduction programmes and food security (Ayeni *et al.*, 2022), bridge the protein deficiency gap (Kone *et al.*, 2022; Udoh *et al.*, 2016 and Owoleke *et al.*, 2016) and serve as an important source of income and employment generation (Olawunmi, 2014). In poor rural households throughout the developing world, it has been recognized that keeping livestock improved nutrition, enhanced economic stability, and lowered gender inequities (World Bank, 2012). Reproductive performance traits such as litter size at birth and weaning are especially important in multiparous

species such as rabbits and pigs (Cartuche *et al.*, 2014) which contribute to economic returns from rabbit rearing (Ragab *et al.*, 2016). Therefore, their improvement can enhance the production of low cost kits. Animal breeding to improve livestock populations utilizes the genetic differences among individuals and achieves improvement through the selection of superior individuals based on the breeding objective (Therkoot, 2019). The purpose of this activity is to increase the frequency of favorable genes in the population, and to prevent genetically poor animals from reproducing.

The genetic improvement of rabbit litter size at birth by artificial selection has been successfully performed (Hill, 2016; Patry, 2018; El-Attrouny and Habashy, 2020 and Blasco *et al.*, 2017). However, failures has also been reported (Szendro *et al.*, 2012; Santacreu, 2019 and Moce and Santacreu, 2010). Furthermore, attempts at improving litter size at birth through selective breeding has led to indirect responses in genetically linked traits. This phenomenon is majorly caused by pleiotropy, a situation whereby one gene influences the expression of more than one trait (Toghiani, 2012). The aim of this study was to improve the litter size at birth in mongrel rabbit does through selection and determine the effect on other birth traits namely: litter weight at birth (LWB), number born alive (NBA) and kits' birth weight (KBW).

MATERIALS AND METHODS

Research Site. This study was conducted at the Rabbitry Unit Agricultural Development Programme (AKADEP), Ikot Ekang, Abak Local Government Area of Akwa Ibom State. Abak is located on latitude 050 58′N and longitude 070 48′E with an altitude of 30m above sea level. The city falls within the rainforest zone of the humid tropics which is characterized by a hot and humid climate. The mean minimum and maximum annual rainfall of 2000-3000 mm, the period of rains being bimodal with a short break in August. Abak has a minimum and maximum temperature range of 280C to 330C with a relative humidity range of between 75 and 90%.

Management of experimental rabbits. Experimental animals were fed concentrate diet containing 15.81% CP, 2480 kcal energy and 8.22% fiber and supplemented with forages (50% *Calopogonium mucunoides* + 50% *Pennisetum perpereum*) in the evening. Forages were wilted for twenty four (24) hours before being fed to experimental rabbits. Concentrate was fed in specially designed aluminium feeding troughs bound to the floor of the hutch with strings. Clean drinking water was served continuously using heavy cement- moulded troughs to prevent tip over. Quantity of feed taken was recorded daily. Experimental animals were housed individually in 3-tier wooden hutches with dimensions 60cm x 90cm x50cm, raised one meter from the floor and fitted with rat and ant proofs. Sides of the hutch were covered with netting to enhance ventilation. All animals were identified with ear-tags and cage labels. Hutches were thoroughly cleaned and disinfected with Izal before stocking and on a monthly basis thereafter. Spent engine oil was applied periodically around the pen to keep away ants. The base population was quarantined for two (2) weeks within which period they were treated with Ivomec at 2mls i.m. per animal and coccidiostat to get rid of internal and external parasites. Routine anticoccidial treatment and prophylactic medications of animals was done monthly.

Reproductive Management. A teaser male was used to identify sexually mature does. Matings were done twice (in the early morning and evening) in the bucks' cages and the dates noted. Gravidity test was carried out on each doe on the 14th day after mating by abdominal palpation. Non-gravid does

were re-mated immediately. For all gravid does, kindling boxes with wooden straw were placed in their cages on day 25 of gestation. Daily checking of nest boxes was done to ascertain kindling and dates recorded. Young rabbits were allowed to remain in the dam's cage until weaning. Weaning was carried out forty nine (49) days post-partum. Cross-fostering was not practiced. All genetic groups of rabbits were subjected to similar environmental, medication and managerial conditions.

Generation of experimental animals. A total of ninety (90) weaned mongrel rabbits, sixty (60) females and thirty (30) males was purchased from reputable farms in Ikot Ekpene, Uyo and Eket senatorial districts of Akwa Ibom State. Thirty (30) mongrel rabbits (10 males and 20 females) were purchased from different farms in each of Abak, Ibiono Ibom and Ikot Abasi representing Ikot Ekpene, Uyo and Eket senatorial districts respectively. This was to ensure the genetic variability of experimental animals by removing any possibility of relatedness and the consequences of inbreeding. Thus, ninety weaned mongrel rabbits constituted the base population (BP). The base population was raised to sexual maturity at five (5) months and mated in a pool in a ratio of one (1) buck to three (3) does which was also used throughout the study. All does mated to a particular buck were noted. Bucks and does from the same senatorial district were not mated. The progeny of the base population constituted the foundation stock (F_S). The F_S was raised to maturity and all data on growth collected. Selected proportion used was 15% for bucks and 35% for does. Bucks were ranked on the basis of their Estimated Breeding value (EBV) for BW16 and the best 15% selected as F1 sires while does were ranked and selected on the basis of their aggregate phenotype or index score (I) resulting from the addition of the estimated breeding value (EBV) for each of the index traits per candidate for selection. The identification numbers of bucks and does mated were noted. Animals in the foundation stock that met the criteria of selection were selected as parents of the next generation (F1). All unselected individuals in the F_S and subsequent generations were culled. Similar procedures were applied to produce F₂ and F₃ population. Cross fostering was not practiced.

Selection of Bucks as Parents. Mass selection was used to select bucks as parents. The selection criterion was body weight at sixteen weeks (BW16). Animals were ranked based on their estimated breeding values (EBV) for the criterion of selection and the best fifteen percent (15%) selected. The expression for determining EBVs from mass selection according to Oldenbroek and VanderWaaij (2014) was as follows:

EBV mass selection =
$$h^2(\overline{P_s} - P)$$
....(1) Where:

 h^2 = heritability of the trait (BW16), $\overline{P_s}$ = mean of selected parents, \overline{P} = mean of parental population.

Selection of Does as Parents. A selection index was used as a selection aid for the mass selection of does as parents. An index predicts the economic value of the offspring of a candidate for selection. The selection index incorporated body weight at first kindling (BWK), litter size at birth (LSB) and litter size at weaning (LSW) as index traits. Females were ranked on the basis of their index scores (*I*) and the best thirty five percent (35%) selected. The index takes the following form:

(I)=
$$a^1h^21x'1 + a^2h^22x'2 + a^3h^23x'3 + a^nh^2nx'n$$
....(2)
Where:

(I) = selection index, a^1 = relative economic weight of body weight at first kindling (BWK), a^2 = relative economic weight of litter size at kindling (LSK), a^3 = relative economic weight of litter size at weaning

(LSW), h^21 , h^22 and h^23 = additive genetic heritability of index traits (BWK, LSK and LSW), x'1, x'2and x'3 = standardized phenotypic values of index traits.

The standardized variable X¹i, was obtained using the following expression according to Becker (1992):

$$X^{1}i = \frac{\overline{Xi} - X^{1}i}{\delta xi}$$
....(3)

Where: xi= record performance of an individual in the ith trait of the index, $X^{1}i=$ mean performance of the whole population in the ith trait of the index, δxi = population phenotypic standard deviation for the ith trait of the index.

Estimation of Heritability (h^2) . Heritability used in the determination of breeding values for candidates of selection was estimated using the Paternal half sib correlation method. Sire variance components estimated using SAS analytical software through Restricted Maximum Likelihood Method (REML) in a nested design was used to derive the additive genetic heritability of traits studied. However, the formula given below according to Willems et al., (2013), was used to calculate the heritability from estimated variance components:

$$h^2s = \frac{4 \sigma^2 s}{\sigma^2 s + \sigma^2 d + \sigma^2 w} \tag{4}$$

where; $h^2s = narrow$ sense heritability, $\sigma^2s = sire$ variance component, $\sigma^2d = dam$ variance component, σ^2 w = error (within progeny variance component).

The Standard errors of heritability (Se h²s) was approximated according to the formula given by Becker, (1992) as follows;

Se h²s =
$$\frac{2(1-t)^2 \{ 1+ (k-1) t \}^2}{k(k-1)(s-1)}$$
(5)

Where:

k = number of offspring per sire, s = number of sires.

Data Collection. Litter traits measured were litter size at birth (LSB), counted on day of kindling as the number of kits born death or alive, litter weight at birth (LWB), measured in grams as the total weight of all kits kindled death or alive, number born alive (NBA), determined on day of kindling as difference between litter size at birth and kits born death and kits' weight at birth (KWB), determined in grams at day of kindling as average weight of all kits kindled.

Predicted Selection Gain (ΔG).

This is amount of gain or progress expected from one generation of selection for a particular trait (Willems et al., 2013). Given by the expression:

$$\Delta G = \overline{(P_S - P)^* h^2}$$
(10)

Where:

 $\overline{P_S}$ = mean of selected individuals for litter size at birth, \overline{P} = mean of population before selection for litter size at birth

 h^2 = heritability of litter size at birth.

Realized (**Observed**) **Genetic Response** (Δ **GR**). This is the response realized in the progeny as a result of selection in the parental generation. Estimated using the expression below:

$$\Delta GR = \overline{x_{ip}} - \overline{x_{iw}} \qquad (11)$$

where: ΔGR = realized genetic response or gain, x_{ip} = mean of progeny of selected parents for litter size at birth, x_{iw} = mean of parental population before selection for litter size at birth.

Experimental Design and Statistical Model The experiment was a Two-Stage Nested or Hierarchical design where dams were nested between sires. The statistical model for analysis of variance is a random effects or linear additive model presented below:

$$Yijk = \mu + \alpha i + \beta ij + \epsilon ijk$$

Where: Yijk = the kth observation of the ith sire and the jth dam, μ = overall mean, α i = random effect of the ith sire, β ij = random effect of the jth dam mated to the ith sire, ϵ ijk = random error \sim iind (0, σ^2).

Data Analysis. Data collected was analyzed through Analysis of Variance for a Nested Design using the Statistical Analysis Systems (2016). Significant effects were separated using Turkey's test.

RESULTS

Phenotypic evolution in litter size at birth (LSB). The response in litter size at birth (LSB) after three generations of selective breeding in heterogeneous rabbits does are summarized in Table 1. The effect of three generations of selection on litter size at birth (LSB) was not significant (p>0.05). The highest (4.51 ± 0.18) and lowest (4.46 ± 0.14) mean values were recorded in F_3 and F_8 , respectively. Intermediate values of 4.50 ± 0.11 and 4.48 ± 0.15 were recorded for F_2 and F_1 generations. It was also observed that although variability was high in the trait (CV% of 19.71, 21.76, 22.39 and 19.70) from F_8 to F_3 respectively, it did not contribute to a significant response as expected with traits having high phenotypic variability.

Table 1. Phenotypic evolution in litter size at birth (LSB) based on generations of selective breeding in heterogeneous rabbit does.

Genetic Group	$\mathbf{F}_{\mathbf{S}}$	$\mathbf{F_1}$	\mathbf{F}_2	F ₃	<i>p</i> Value	sig
LSB (no.)	4.46±0.14	4.48±0.15	4.50±0.11	4.51±0.18	0.8892	ns
CV%	19.71	21.76	22.39	19.70		

LSB = litter size at birth, F_S = foundation stock, F_1 = first generation, F_2 = second generation, F_3 = third generation, CV%= coefficient of variation, Sig= significant response, ns= not significant at α =0.05.

The non-significant (p>0.05) evolution in LSB from F_S to F_3 obtained in our study is supported by Ziadi (2012) who observed that in rabbits, direct selection for litter size was less successful than

expected and that in prolific species such as rabbits and pigs, direct selection for litter size did not achieve expected objectives respectively. However, this result does not agree with El-Attrouny and Habashy (2020) that generations of does selected on litter size at birth were significantly superior in the trait (difference of 1.62 kits) when compared with the base population. The difference between this study with El-Attrouny and Habashy (2020) could have been occasioned by number of generations of selection. The marginal but consistent improvement in LSB as selection progressed could have been due to the selection applied on the trait which agrees with Patry (2018) that selective breeding positively influenced the performance of the domestic rabbits. The marginal improvement in LSB obtained in present study could be attributable to the low heritability estimates of the trait which is confirmed by Wheelwright et al. (2014) that traits associated with reproductive fitness were characterized by low estimates of genetic parameters. Cartuche et al. (2014) in their study reported that such traits would produce low selection responses and a difficulty of improvement through selective breeding respectively. The coefficient of variation values of 19.699- 22.388 recorded in this study which indicated a high variability in the trait between individuals in the population is higher than 18.4% obtained by Montes-Vergara et al. (2021) for LSB in New Zealand White rabbits but lower than 47.2% noted by Elamin and Yousif (2011) for total born (TB) in local rabbits of Sudan. Disparity between values could be on account of differences in population size with its concomitant effect on genetic variation (Rutherford et al., 2019; Hare et al., 2011; Palstra and Ruzzante, 2008). The values in LSB $(4.46 \pm 0.14 - 4.51 \pm 0.18)$ obtained in this study agrees with value of 4.49 ± 1.15 reported by Khan et al. (2018) in local Pakistani rabbits, close to 4.07 ± 1.43 kits reported by Okoro et al. (2012) in unselected non-descript rabbits in South Eastern Nigeria but disagrees with higher values of 6.51± 0.47 and 6.24 ± 0.63 in New Zealand White and crossbred rabbits respectively recorded by Akinsola et al. (2013) and 5.9 ± 0.37 - 7.1 ± 0.37 kits obtained in purebreds by Juarez et al. (2021) in Spain. Variation in results between present study and the authors may be attributed to differences in genotype, location and management, genotype being a known significant source of variation in rabbit litter traits (Juarez et al., 2021). Furthermore, the result in this study agrees with the report of Montes-Vergara et al. (2021) that the differences in number of kits born in rabbits may be due to maternal effects such as conception rate, number of matured oocytes implanted and nurtured to term by the dam.

Selection Response in Litter Size at Birth. The predicted responses for litter size at birth (LSB) were 0.02, 0.03 and 0.03 while realized responses were 0.02, 0.02 and 0.01 in F_1 , F_2 and F_3 generation kits respectively (Table 2). Realized responses were lower than expected in F_2 (0.02 and 0.03) and F_3 (0.01 and 0.03) generation kits but similar (0.02 and 0.02) in F_1 generation kits. F_1 and F_2 recorded the highest values for realized response which were however similar while F_3 recorded the least.

Table 2. Estimates of predicted and realized selection response in litter size at birth of heterogeneous rabbit does in three generations of selective breeding

Response	Generations of Selection				
	\mathbf{F}_1	$\mathbf{F_2}$	\mathbf{F}_3		
Predicted(g)*	0.02	0.03	0.03		
Realized(g)**	0.02	0.02	0.01		

^{*=} Parent Generation, **= Progeny Generation, F₁= First Generation, F₂= Second Generation, F₃= Third Generation.

The low realized selection responses observed across generations for LSB (Table 2) is in agreement with findings by Szendro *et al.* (2012) who recorded low selection responses per generation (0.03 to 0.18) in rabbit litter size at birth. This result could have been due of low heritability for the trait stipulating that the trait was more in the control of the environment than additive gene effect. In agreement with this

report, Agaviezor and Ologbose (2020) reported that selection on litter size in prolific species such as rabbits and pigs was not successful due low heritability. The reduction in realized response from 0.03 in F_2 to 0.02 in F_3 could have been due to changes in the genetic variance during selection or the influence of modifier genes. This result agrees with Szendro *et al.* (2019) that variation in the proportion of the genetic variance influenced the response to selection and Argente *et al.* (2016) that gene modifiers could prevent the full expression of phenotypes

Correlated response in birth traits of heterogeneous rabbits.

Correlated response in birth traits of heterogeneous rabbit does in three generations of selective breeding on litter size at birth (LSB) is presented in Table 3. The correlated effect of mass selection on litter size at birth on the litter birth weight (LBW), number born alive (NBA) and kits' birth weight (KBW) of heterogeneous rabbits was not significant (p>0.05). However, numerical increases were noted in LBW and NBA but decrease in KBW

Table 3. Correlated response in birth traits of heterogeneous rabbit does in three generations of selective breeding on litter size at birth (LSB).

Traits	FS	F1	F2	F3	pValue	sig.
LBW(g)	193.82±2.03	193.38±2.27	203.79±2.83	203.81±3.87	0.0770	ns
KBW(g)	43.56±1.16	43.36±1.41	42.99±0.65	42.91±0.94	0.5226	ns
NBA(no.)	4.09±0.16	4.11±0.13	4.19±0.09	4.21±0.17	0.8329	ns

k, F1= first gene- ration, F2 = second generation, F3= third generation, SR= significant response, LWB= litter weight at birth, KWB= kit weight at birth, NBA= number born alive, ns= not significant at α =0.05.

Correlated response in litter birth weight (LBW). The non-significant (p>0.05) improvements in LBW observed in present study with selection for LSB compares with El-Attrouny and Habashy (2020) who noted correlated superiority of selected generations on litter birth weight of New Zealand White (NZW) rabbits selected on litter size at birth and with Hine *et al.* (2014) who recorded higher LBW in rabbits with higher litter sizes. This was expected because LBW constitutes the weight of all kits born (dead or alive)-hence the more kits born, the higher the LBW. The numerical increases in LBW with LSB recorded in this study is indicative of a positive genetic correlation between the traits. This result agrees with O'Neal and Knowles (2018) who observed a positive genetic correlation (0.69) between litter size and litter weight at birth in rabbits. The values of $193.21\pm1.60-203.81\pm0.56g$ recorded for LWB in present study is within the range of 185.58 to 266.58 g observed by Rogers (2023) in mixed breed rabbits but lower than $425\pm134-577\pm154$ g published by Oguike and Okocha (2019) in crosses between an Algerian synthetic line and a Spanish INRA2666 strain. The breed and nutrition of rabbits under study and general management could have accounted for the disparity in results.

Response in Kits' Birth Weight (KBW). The reduction in kit weight at birth of mongrel rabbits as selection on LSB progressed obtained in current study is in order with observations by Manchini *et al.* (2014) that body weights of kits decreased as litter size increased both at birth and at weaning such that kits in smaller litter groups had the highest individual body weight of 148.75g± 39.42 whereas kits belonging to largest litter size of seven had the least individual birth weight of 69.93g±0.93. Several authors (Hanaa *et al.*, 2014; Olateju and Chineke, 2022 and Gambo *et al.*, 2020) have recognized that increase in the number of rabbits per litter caused a decrease in the average weight of kits at birth. Furthermore, this study agrees with observations by Szendro *et al.* (2012) that the growth of fetuses during gestation were affected by their number, position in the uterine horn and the number of blood vessels supplying each fetus. The decrease in KWB as litter size increased recorded in our study, could

have been due to a higher intra-uterine competition both for space and nourishment between the fetuses. Hence, kits in larger litters develop less rapidly than those of smaller litters, resulting in lower birth weight. This report agrees with findings by Manchini *et al.* (2014) that heavier birth weight of kits in smaller litter sizes was contributed by the sufficient intra-uterine nourishment available for development.

The decline in KWB could have resulted from a negative genetic correlation between the traits, meaning that KWB and LSB were controlled by the same additive genes but in opposite direction, which confirms findings by Lenoir *et al.* (2012) that there is a strong negative genetic correlation (-0.70) between litter size at birth and kits' weight at birth in New Zealand White, Carlifornian, Palomino Brown and Havana Black rabbits raised in the humid tropics. Furthermore, our study agrees with reports by Ayoola *et al.* (2016) that rabbits with least litter sizes showed highest KWB across the weeks considered. The KBW values recorded in present study (42.91 – 43.55g) is within the range of 38.00-55.71g published by Fadare and Fatoba (2018) in a heterogeneous population of rabbits and 38.42-48.16 g recorded by Adeolu *et al.* (2019), but lower than $57.97 \pm 1.41g - 59.66 \pm 0.92g$ observed in New Zealand White and New Zealand White x Carlifornian crosses by Akinsola *et al.* (2013). Disparity in result could be attributed to differences in the breed of animals used. A significant breed effect on KBW had been documented by Fadare and Fatoba (2018)).

Response in Number Born Alive (NBA). The non-significant (p>0.05) indirect effect of mass selection for LSB on NBA in present study is comparable to observations by Adeolu *et al.* (2019) who found positive correlated responses on NBA and LSB after eleven generations of selection on litter size at birth in rabbits. The slight increases in NBA as selection for LSB advanced was a consequence of the increase in LSB and positive genetic relationship between the traits which agree with Toghiani (2012) that selection for one trait would increase the other if the genetic correlation was positive and decrease if it was negative and Adeyemo *et al.* (2018) that genetic correlations were a measure of genetic factors shared between two traits.

The highest value in NBA (4.21 kits) and LSB (4.51 kits) noted in F_3 which was the last generation for selection in LSB showed that these traits could not evolve independently due to shared genes. This result agrees with Bolet *et al.* (2012) that genetic correlation limits independent evolution of linked traits and that co-evolution will depend on the magnitude of the genetic correlation. Values of $4.09 \pm 0.11 - 4.21 \pm 0.30$ kits for NBA obtained in present study agrees with 4.13 ± 0.22 kits published by Elamin and Yousif (2011) in local Sudanese rabbits but lower than 8.13 ± 3.42 kits and 8.29 kits obtained by Badawy *et al.* 2019) in a synthetic line of rabbits and (Sodini *et al.*, 2018) in rabbits selected for litter size at weaning respectively. Breed of rabbits could have accounted for the variation in results.

CONCLUSION

It could be concluded from this study that litter size at birth in mongrel rabbits did not significantly evolve over three generations of mass selection although observed responses were more than expected in all generations. Birth traits evaluated alongside such as litter birth weight, number of kits born alive and kits' birth weight were not significantly influenced by mass selection on the criterion, although numerical increases in LBW and NBA and decrease in KBW were recorded. It is therefore recommended that genetic improvement approaches in litter size at birth maybe better served with other methods such as crossbreeding than mass selection.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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