OPTIMISATION OF N-ETHYL-N-NITROSOUREA (ENU) DOSE FOR MUTAGENESIS IN THE NIGERIAN YORUBA ECOTYPE CHICKEN

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ABSTRACT

The optimal dose and regime of ENU for mutagenesis in the chicken was determined through dose titration (0mg/kg body weight, control group; 100mg; 200mg; 300mg and 400mg/kg body weight) and regime variation (single shot, half dose in each of two consecutive weeks, and 1/3 dose in each of 3 consecutive weeks) studies in one hundred and twenty (120) 25 week-old improved Nigerian local chicken cocks. The study was laid out as a 5 X 3 factorial layout of treatments within a completely randomized experimental design. Eight individually tagged cocks were assigned to each treatment. Mortality was recorded over 48 hours from the point of each ENU administration. From week 5 -14 following completion of the assigned ENU dose/regime, semen was collected from each ENU mutagenized cock twice weekly and used to inseminate two Issa Brown commercial layers hens i.e. 1:2 mating ratio and a total of 240 hens used. Eggs were collected over a period of 5 days in each week and set in an egg incubator to test fertility (candling at day 12). The data showed that ENU dose and regime independently and significantly ($p<0.05$) control fertility. The 400mg dose produced significantly lower fertility than the control group throughout the examined period, indicating an absence of recovery of fertility to levels comparable to the Control group. There was transient loss of fertility in the 300mg dose group relative to the control group in weeks 6, 8, 10, 11 and 13 and at week 14, fertility was not significantly ($p>0.05$) different from the control group. The 200mg dose showed no significant loss of fertility throughout the experimental period, and this was mirrored in the 100 mg group except at week 10. ENU regime significantly ($p<0.05$) determined fertility at weeks 7 and 9 - 11 only. Administration of 1/3 dose in each of 3 consecutive weeks resulted in the longest period of consistent loss of fertility (weeks 9 – 11) relative to the group given the assigned dose in a single shot regime. Cumulative mortality within 48 hours after ENU administration was significantly determined by dose (0mg, 100 mg, 200 mg < 300 mg < 400mg) and regime (1 dose > 2 doses, 3 doses). The 300 mg dose administered in 3 equal fractions over 3 weeks resulted in transient sterility and recovery and is therefore recommended for ENU in Nigerian local chicken.

Keywords: Yoruba Nigerian Local Chicken, Mutagenesis, N-Ethyl-N-Nitrosourea.

INTRODUCTION

Use of Ethyl-N-Nitrosourea (ENU) mutagenesis to develop an allelic series of Yoruba Nigerian Local Chicken (Y-NLC) growth rate gene determinants will reveal the genetic architecture of trait determination and markers that are useful for trait improvement through Marker assisted Selection. Successful use of the ENU-based strategy requires preliminary determination of optimal doses for mutagenesis in the chicken, and specifically in the breed of interest (i.e. Y-NLC). The present study determined the optimal dose and regime of ENU for mutagenesis in the chicken, guided by established levels in the most widely studies model, the Laboratory Mouse (Russel *et al.*, 1979; Hitotsumachi *et al.*, 1985; Nolan *et al.*, 2000 and Toye *et al.*, 2004)

MATERIALS AND METHODS

Experimental Design: A 3×5 factorial layout of treatments within a Completely Randomized experimental design was used to investigate the effect of 3 regimes of administration of 5 dosage

levels of ENU on fertility/sterility and survival/mortality in Y-NLC cocks.

Birds: One hundred and twenty (120) wing-tagged Yoruba Nigerian Local Chicken (Y-NLC) cocks were randomly distributed at 25 weeks of age into 15 groups of 8 cocks. Two hundred and forty (240) hens were randomly distributed into 120 battery cages such that there were 2 hens per cage.

Preparation of ENU and Sham (-ve Control) stocks: Fresh stocks of ENU were prepared prior to each weekly intraperitoneal injection of cocks. ENU was dissolved in 95% ethanol to 2.5 g ENU/6.25 ml (400mg ENU/ml; 10x working stock concentration) and diluted with 0.1M phosphate buffer (pH 6.0) at ratio 1:9, yielding a working stock (x1) concentration of 40 mg ENU/ml. Sham stocks were prepared by mixing 2.5 ml Normal Saline with 3,75 ml 95% ethanol; 10x sham stock concentration) and diluted with 0.1M phosphate buffer (pH 6.0) at ratio 1:9, yielding a Sham stock (x1) containing 0 mg ENU/ml.

N-Ethyl-N-Nitrosourea (ENU) dose/regime (D/R) groups: Fifteen, ENU (Sigma-Aldrich, UK) dose titration (0 mg/kg body weight, control group; 100mg/kg body weight; 200mg/kg body weight; 300mg /kg body weight and 400mg/kg body weight) and regime variation (single shot, half dose in each of two consecutive weeks, and 1/3 dose in each of 3 consecutive weeks) groups were examined, namely: (1) 0 mg/kg body weight ENU dose administered in one shot (0D/1R) i.e. sham group 1; (2) 0D/2R sham group 2; (3) 0D/3R sham group 3; (4) 100D/1R; (5) 100D/2R; (6) 100D/3R;(7) 200D/1R; (8) 200D/2R; (9) 200D/3R; (10) 300D/1R; (11) 300D/2R; (12) 300D/3R; (13) 400D/1R; (14) 400D/2R; (15) 400D/3R.

Administration of ENU: One hundred and fifty cocks were randomly assigned to each of the 15 D/R experiment groups such that there were 8 cocks/group. Each cock was weighed and injected (i,p.) with the assigned ENU dose in the assigned regime.

Mortality Records: Mortality within each treatment group was recorded over a 48 hour period immediately following each administration of ENU (i.p.) and summed up over the assigned regime.

Fertility Trial: From week 5 -14 following completion of the assigned ENU dose/regime(The time window examined mirrors that which is routinely used in mouse studies; Nolan *et al.*, 1998), semen was collected from each ENU mutagenized cock twice weekly and used to inseminate two pair-housed Issa Brown commercial layers hens (1:2 mating ratio and a total of 240 hens) by use of the artificial insemination technique of Burrows and Quinn (1937). Eggs were collected over a period of 5 days in each week and set in an egg incubator to test fertility. At the 12th day of incubation, eggs were candled to test for fertility.

Statistical Analysis

The data on fertility was subjected to analysis of variance as appropriate for a factorial layout of treatments by use of SPSS version 22 (SPSS, 2013) and Duncan's multiple range tests. The survival data was subjected to chi-squared analysis.

RESULTS AND DISCISSION

Table 1 shows the effect of ENU dose and regime of administration on day 12 fertility of incubated eggs. The 400 mg/kg body weight dose of ENU resulted in significant ($p< 0.05$) loss of fertility in the cocks following ENU administration, with an average fertility of 9.69% of the level in the Control group. When compared to the control group with an average fertility of 61.65%. The groups injected with 300 mg/kg body weight of ENU had a significant loss of fertility in week 6, 8, 10, 11 and 13 of the fertility test with an average loss of fertility of 53.25% of the control and regained fertility at week 14. The 200mg/kg body weight ENU group showed no significant loss in fertility. In the 100mg/kg body weight group, there was no significant loss of fertility except in the 10th week in which the fertility percent of 35 ± 7.64 was significantly lower than that of the control (61.74 ± 7.43).

Table 1 also shows the effect of various weekly regime of administration of ENU in Improved Nigerian local chickens. It was shown that there were no significant effects of weekly attenuated dosing of ENU except in weeks 7, 9, 10 and 11 where there were significant differences ($p<0.05$) in fertility among the three regimes. In weeks 9,

10 and 11 the groups injected with 3 weekly attenuated doses of ENU showed a consistent significant loss of fertility of 32.48%, 49.32% and 37.04% relative to the groups injected with once weekly dose.

Table 2 shows the effect of various ENU doses and regime of administration on mortality rate of improved local Nigerian Chickens. The 400mg/ kg body weight dose resulted in about 46% mortality as a result of the injected ENU, while the 300mg/kg body weight dose had 25% mortality. The control group, 100mg and 200mg doses did not result in any mortality post injection. The effect of the regime of administration of ENU on mortality of improved local Nigerian Chicken was also shown in table 2. The regime of once weekly dose had the highest mortality rate of 32.5 % while the birds given attenuated doses of twice weekly and thrice weekly resulted in 5 % mortality. Figure 1 shows the growth pattern of the improved local Nigerian chicken and the Marshall strain of the broiler breeder parent stock. There was a disparity of 60% and 39.5% in absolute weight in week 4 and 8 respectively with the Marshall breeder attaining live body weight of 1240 grams at 8 weeks compared to the local birds with average live body weight of 750 grams at 8 weeks.

The variation that exists between the Marshall breeds of boiler chicken and the Yoruba ecotype improved Nigerian local chicken (Fig. 1) indicates the prospect for the improvement of early growth in the later. It also confirms the possibility of using these divergent populations in genetic mapping studies like the current one.

The mutagenic and cytotoxic effects of ENU vary depending on the dosage regimen and strain of animal used (Hitotsumachi *et al.*, 1985; Davies *et al.*, 1999). The high mortality of birds injected with 400mg/kg body weight of ENU indicates the high cytotoxic effects of ENU at this dosage regimen. It was observed that the 300mg dose had high mortality however administering the dose at a fractionated regime of three weekly doses reduces the cytotoxic effect with no mortality recorded. The temporary loss of fertility in the male mouse injected with ENU is indicative of successful mutagenesis (Nolan *et al.*, 1998). The result from this study reveals that giving the improved local Nigerian chicken ENU doses of 100mg and 200mg at either single shot dosing regimen or fractionated doses will not induce mutation as there were no significant loss of fertility. It was also evident that administering ENU at a dose regime of 400mg/kg body in the local chicken resulted in permanent loss of fertility with the cocks not recovering from their sterility throughout the experimental period. The 300mg however resulted in loss of fertility during the experimental period and significant recovery from sterility at 14th week. The regime 3 of administration in which ENU was injected intraperitoneally as 3 fractionated doses over a 3 week period gave a consistent loss and recovery of fertility.

CONCLUSION

It is concluded from this study that ENU induces streility in chicken. The injection of ENU at 400mg / kg body weight is toxic and highly lethal. When administered at 3 weekly fractionated doses, 300mg / kg body weight is effective in inducing transient sterility without resulting in mortality. In furtherance of this study, a cohort of F1 progeny of Improved Nigerian Local Chicken cocks mutagenised by use of 300mg ENU dose/ kg bodyweight administered in a regime of 3 equal fractions over 3 weeks is now being screened for phenovariations to identify an allelic series of putative-dominant growth gene mutants/outliers.

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Table 1: Table 1: Effect of Various doses and regime of Administration of N-Ethyl-N-Nitrosourea (ENU) on Fertility (±SEM) of improved Nigerian Local Chicken Cocks for 10 weeks post injection

		Weeks									
Main Effects		5	6	7	8	9	10	11	12	13	14
	Doses of ENU:										
Dose	0 mg	50 ± 7.52^{bc}	65 ± 8.01^{c}	60 ± 6.53^{b}	67.83 ± 5.59^{c}	60.87 ± 7.17^{bc}	61.74 ± 7.43^{c}	61.74 ± 7.54^{c}	54.78 ± 8.36^{b}	65 ± 7.96^{c}	69.57 ± 7.94^{b}
	100 mg	45.83 ± 8.21^{bc}	53.33 ± 7.86^{bc}	46.67 ± 7.86^{b}	50 ± 6.7^{bc}	38.33 ± 7.89^{b}	35 ± 7.64^{b}	45.83 ± 7.27^{bc}	33.33 ± 6.77^{ab}	41.67 ± 8.16^{bc}	53.91 ± 8.09^{b}
	200 mg	60.83 ± 7.17^{c}	58.33 ± 8.25^{c}	57.5 ± 8.19^{b}	49.17 ± 7.02^{bc}	70 ± 7.61^{c}	55 ± 7.25^{bc}	49.17 ± 8.6^{bc}	53.33 ± 8.31^{b}	40.83 ± 8.38^{bc}	54.17 ± 8.03^{b}
	300 mg	32.22 ± 8.73^{b}	32.22 ± 9.02^{b}	42.22 ± 9.55^{b}	37.78 ± 8.69^{b}	35.56 ± 8.94^{b}	33.33 ± 9.43^{b}	31.11 ± 8.28^{ab}	37.78 ± 10.4^{b}	36.67 ± 8.89^{b}	45.56 ± 9.26^{b}
	400 mg	3.08 ± 3.08^{a}	4.62 ± 3.32^{a}	15.38 ± 7.56^{a}	13.85 ± 7.3^{a}	6.15 ± 6.15^{a}	9.23 ± 6.65^{a}	12.31 ± 7.35^{a}	9.23 ± 6.25^{a}	7.69 ± 4.26^{a}	15.38 ± 8.52^{a}
	Regime of Administration:										
Regime	Regime_1	55.38 ± 7.71	65.38 ± 8.19	53.08 ± 7.76^{b}	46.92 ± 6.27	57.69 ± 7.39^{b}	62.31 ± 7.63^{b}	57.69 ± 7.47^{b}	40 ± 6.84	49.81 ± 7.76	59.2 ± 7.16
	Regime_2	37.89 ± 7.03	40 ± 6.75	30.81 ± 5.73^{a}	47.57 ± 6.45	44.32 ± 7.07^{ab}	37.3 ± 6.49^{a}	38.92 ± 6.43^{a}	46.49 ± 7.06	35.14 ± 6.55	48.11 ± 6.99
	Regime_3	37.89 ± 5.27	41.05 ± 5.98	58.95 ± 5.94^{b}	46.32 ± 5.6	38.95 ± 6.26^{a}	31.58 ± 5.31^{a}	36.32 ± 5.93^{a}	34.21 ± 6.53	42.11 ± 6.7	47.37 ± 6.68
	Interaction Effect:										
	Dose x Regime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means on the same column with different superscript ^{abc} are significantly different (P< 0.05)

Table 2. Effect of Dose and Regime of N-Ethyl-N-Nitrosourea (ENU) on Mortality of improved Nigerian Local Chicken Cock

Factor	Birds (N)	Live (N)	Dead (N)	Mortality (%)
Dose (mg ENU/kg body weight)				
0	24	24	0	0^a
100	24	24	0	0^a
200	24	24	0	0^a
300	24	18	6	25^b
400	24	13	11	45.83^c
Regime of Administration				
1	40	27	13	32.5^b
2	40	38	2	5^a
3	40	38	2	5^a

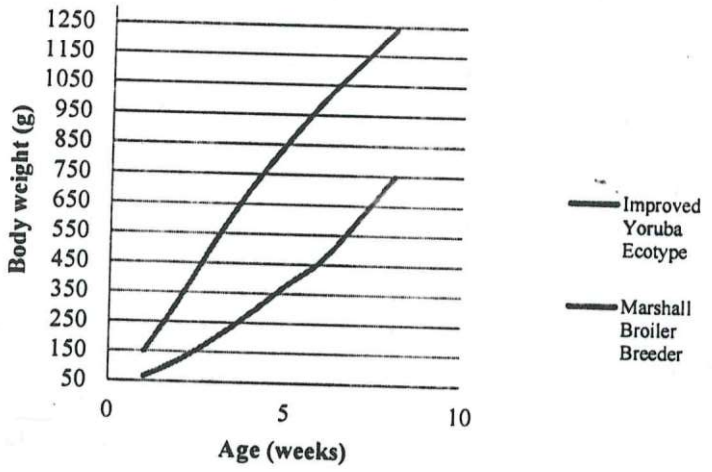


Figure 1. Body weight of Marshall broiler and Improved Yoruba Ecotype Nigerian Local Chicken from age 1 – 8