

1 Effects Of Incision Variation Length On *Heterobranchus bidorsalis* Male Spawners To 2 Extract Milt For Induced Breeding.

3 4 Abstract

5 Matured African catfish (*Heterobranchus bidorsalis*), size ranging from 1.40-2.00 kg average
6 total body weight (TBW) were procured from a private fish farm and transported in
7 perforated 50 litre water holding capacity jerry can to the hatchery and maintained for 2
8 weeks. They were fed with 40 % crude protein commercial diet with good water quality
9 management before being used for breeding. Incision at variation length 2.50, 3.40 and 4.00
10 cm were made on the abdominal region (gonad position) of the male spawners after
11 anaesthetic treatment using sterilized surgical instrument to extract milt to fertilize egg. The
12 incised position was sutured using simple interrupted suture pattern with catgut chromic 2/0
13 stitch. The spent spawners after operation survived on gradual healing and recuperation
14 within 14 days post- surgery. Fecundity, volume of milt extracted, percentage fertility and
15 hatching differed significantly ($P<0.05$) among treatments. Treatment 3 (3.40 cm) was the
16 most effective incision length that gave best results in term of percentage hatching (92.04^a),
17 weight gain (28.37), specific growth rate (2.36) and percentage survival (68.77 ± 4.224^a) of the
18 bred hatchlings as compared to other treatments. The male spawners of *H. bidorsalis* could be
19 incised (cut) on abdominal region (gonad position) at length of 3.40 cm to effectively extract
20 milt to fertilize egg without adverse effect on the fish to enhance fingerling production.

21 **Key words:** Incision, milt, *H. bidorsalis*, recuperation and induced breeding.

22 23 Introduction

24
25 Aquaculture presently is one of the fastest growing food production systems in the world with
26 developing countries contributing significantly [1]. Over the years there has been stagnating
27 yields from many capture fisheries due to attendant reasons such as environmental unfriendly
28 practices by man and challenge of climate change. There is increasing demand for fish and
29 fishery products, high expectation for tremendous contribution to the world's aquatic food
30 production. This in no small measure will help in contributing to food security and alleviate
31 poverty particularly in developing countries [2]. Aquaculture practice is significant in
32 nutritional, social, economic and environmental aspects of human endeavour; hence the need
33 for further development and expansion of the sector and effort for sustainable development is
34 important [3].

35 Fish is known to be one of the cheapest sources of protein for human consumption globally
36 especially in areas where cattle and beef production is expensive [4]. According to [5] the

37 demand for fish protein is rising and there is need to increase production of food including
38 fish to feed human population. Fish protein has many advantages over beef, mutton and pork
39 as it supplies essential nutrients such as omega-3 fatty acids and iodine which are lacking in
40 other animal proteins [6].

41 One major constraint to aquaculture development in developing countries is inadequate
42 supply of good quality fish seed to stock culture enclosures. Successful aquaculture begins
43 with stocking adequate and good quality fingerlings in ponds with optimum conditions that
44 enhance rapid growth and harvest within short possible time [7]. For maximum production,
45 especially for commercial purpose, the fish farmer has to obtain adequate number of
46 fingerlings to meet the production target.

47 [8 stated that fish breeders need to make use of brood fish that is viable with good vigour and
48 disease free and disease resistant strain that produces healthy progeny. In Carp species milt in
49 mature males are released by a slight press on the abdomen and semen hand stripped on to
50 the eggs. This is not possible in African male catfish species hence poses a great challenge to
51 fish breeders because they cannot be handled in like manner. The slight press on the abdomen
52 of male African catfish (*H. bidorsalis*) spawners releases milt from gonads into lobes of the
53 seminal vesicles and not directly through the genital papilla [9].

54 Male African catfish (*H. bidorsalis*) therefore has to be sacrificed and have their gonads
55 removed to squeeze out milt to fertilize egg. In view of the above, this research was
56 conducted to determine the appropriate length of incision on catfish *H. bidorsalis* male
57 spawners to extract milt to fertilize egg without killing the fish.

58 **Materials and Method**

59 Twenty four (24) matured brooders (12 males and 12 females) of *H. bidorsalis* ranging from
60 1.40-2.00 kg Total Body Weight (TBW) were procured from a private fish farm and
61 transported in perforated 50 litre water holding capacity jerry can to the hatchery. On arrival,

they were disinfected with 5 % salt bath for 5 minutes as described by [1] and then acclimatized for 2 weeks. They were maintained under optimum temperature, good water quality management at optimum required limit for fresh water fish culture. They were fed adequately with 40 % crude protein commercial diet. After acclimatization the brooders were carefully selected and treated with a single dose of hormone (Ovaprim) 0.5 ml/kg body weight according to the method of [10]. Injection was given intraperitoneally. Eggs were stripped into a clean plastic bowl from injected females after latency period of about eleven hours at water temperature of between 25-32⁰C. Fecundity was determined as thus:

$$\text{Fecundity} = \frac{\text{Total weight of Stripped Eggs}}{\text{Weight of Sub-Sample}} \times \text{Total Number of Eggs in Sub-Sample}$$

About 9.5 g of total stripped eggs was use for fertilization.

Six males were sacrificed to remove testis and squeezed out to obtain milt to fertilize egg.

Surgical operation was carried out on the remaining males using sterilized surgical instruments at the dorso-ventral part of abdominal region, location of the testis. The fish were injected with general anaesthesia Ketamine Hcl at dose of 0.1 ml/kg body weight before operation. Transparent metre rule was used to take measurements of 2.50, 3.40 and 4.00 cm long at ventral side of the abdomen to determine the appropriate and exact length to cut or incise that would expose the testes good enough to extract milt. Four treatments was considered as control (T1: 0.00 cm); T2 (2.50 cm); T3 (3.40 cm and T4 (4.00 cm) and each replicated three times.

The fish were placed dorsally on a wet disinfected white clean cloth spread on clean table with the head covered with a piece of wet clean towel. The surface of the abdomen was disinfected with methylated spirit (40 %) before incision was made on the ventral side. The incision was extended towards the head with sterilized surgical scissors 3-5 cm long for all

87 replicates with the aid of 2 assistants to expose the internal organs including testes which
 88 remained intact. The digestive tracts were pushed aside to reveal the testes. Milt was
 89 extracted from the testes using sterilized 2 ml size syringe and needle. Milt volume was
 90 weighed and then observed under Binocular Olympus Microscope to determine their motility
 91 before being used to fertilize eggs. The incised spot was sutured using simple interrupted
 92 suture pattern with catgut chromic 2/0 stitch. The surgery on each fish lasted for about 30
 93 minutes. Each incised fish was placed in a plastic trough with well aerated fresh water to
 94 enable them recover. After recovery, each fish was kept in a separate concrete tank (2 m x 2
 95 m x 1 m) length, width and depth respectively. This contained broad spectrum antibiotic
 96 (Dav-Biotic) at a dose of 5 g/L. The fish were there for 8 days without food under intensive
 97 care and monitored for healing, recuperation and recovery. The surgery and milt collection
 98 were performed in three replicates. The milt and eggs were mixed together gently with plastic
 99 spoon for 2-3 minutes. Small quantity of saline solution was then poured onto the fertilized
 100 eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and
 101 introduced into the incubators. Incubation was monitored for 24-48 hours with gentle water
 102 flow through in which aeration was maintained using aquarium electric pump.

103

104 Water quality parameters such as Dissolved Oxygen (DO), pH, temperature, conductivity and
 105 ammonia were monitored and measured fortnightly during incubation and rearing of the
 106 hatchlings. Percentage fertility, hatching and survival were determined according to method
 107 described by [11] using the formulae:

$$\begin{aligned}
 108 \quad \text{Percentage fertility} &= \frac{\text{Number of fertilized eggs}}{\text{Number of eggs stripped}} \times 100 \\
 109
 \end{aligned}$$

$$\begin{aligned}
 110 \quad \text{Percentage hatching} &= \frac{\text{Number of fry hatched}}{\text{Number of fertilized eggs}} \times 100 \\
 111
 \end{aligned}$$

112 Percentage survival = $\frac{\text{Cumulative survival}}{\text{Total number stocked}} \times 100$

113

114 Specific Growth Rate (SGR) was determined using the method as described by [12]. $\text{SGR} =$

115 $\frac{\text{Log } W_2 - \text{Log } W_1}{T_2 - T_1} \times 100$

116

117 Where W_1 = weight at time 1, W_2 = weight at time 2, T_1 = Time one, T_2 = Time two and Log

118 = Natural logarithm.

119 Results obtained were analyzed by One Way Analysis of Variance (ANOVA) while means

120 were compared for significant differences at 0.05 significant levels using Duncan's multiple

121 range tests using SPSS version 15.0.

122

123 **Results**

124 The results in Table 1 shows fecundity, volume of milt extracted, percentage fertility and

125 hatching. Treatment 3 had highest fecundity and differed significantly ($P < 0.05$) from other

126 treatments. Volume of milt extracted in treatment 1 (control) differed significantly ($P < 0.05$)

127 as compared to other treatments. Similarly, percentage fertility and hatching in treatment 4

128 and 3 respectively differed significantly ($P < 0.05$) as compared to other treatments. The

129 results in Table 2 shows Incision Variation Lengths (IVL) (2.50, 3.40 and 4.00 cm) long

130 made on the dorsal ventral side of the abdomen on the position of testes. The variation in

131 incision length was to determine the best length that could reveal the testes properly to extract

132 milt without stressing the fish. Mortality of male spent spawners was recorded at 3rd and 6th

133 day post-surgery as shown in plates I and II. The results of gradual and progressive healing

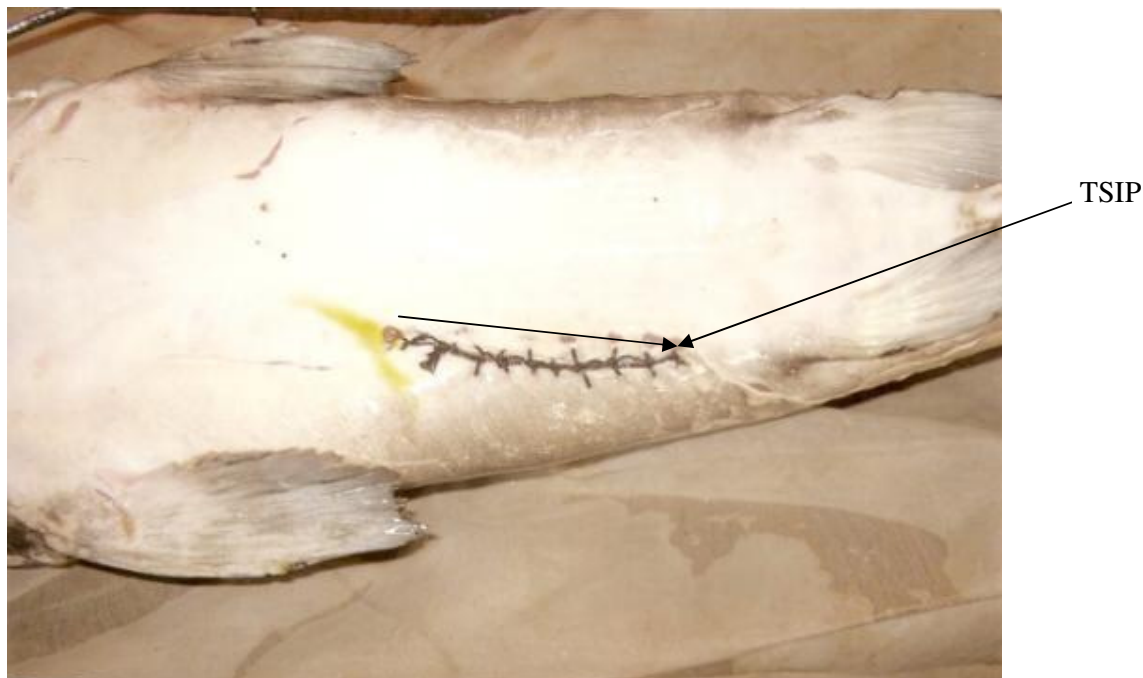
134 and recuperation process of the incision point at various time intervals are presented in plates

135 III. The results in Table 3 shows growth performance of *H. bidorsalis* bred fingerlings

136 through induced breeding of incision variation length method for 12 weeks. Treatment 3 had

137 highest mean length gain, mean weight gain and specific growth rate of 6.88, 28.37 and 2.36
 138 respectively. Similarly, the results in Table 4 shows grand cumulative mean
 139 mortality/survival percentages of *H. bidorsalis* fingerlings produced through induced
 140 breeding methods of incision variation length and reared for 12 weeks. It reveals that
 141 treatment 3 had the highest survival of 68.77 % bred *H. bidorsalis* fingerlings.

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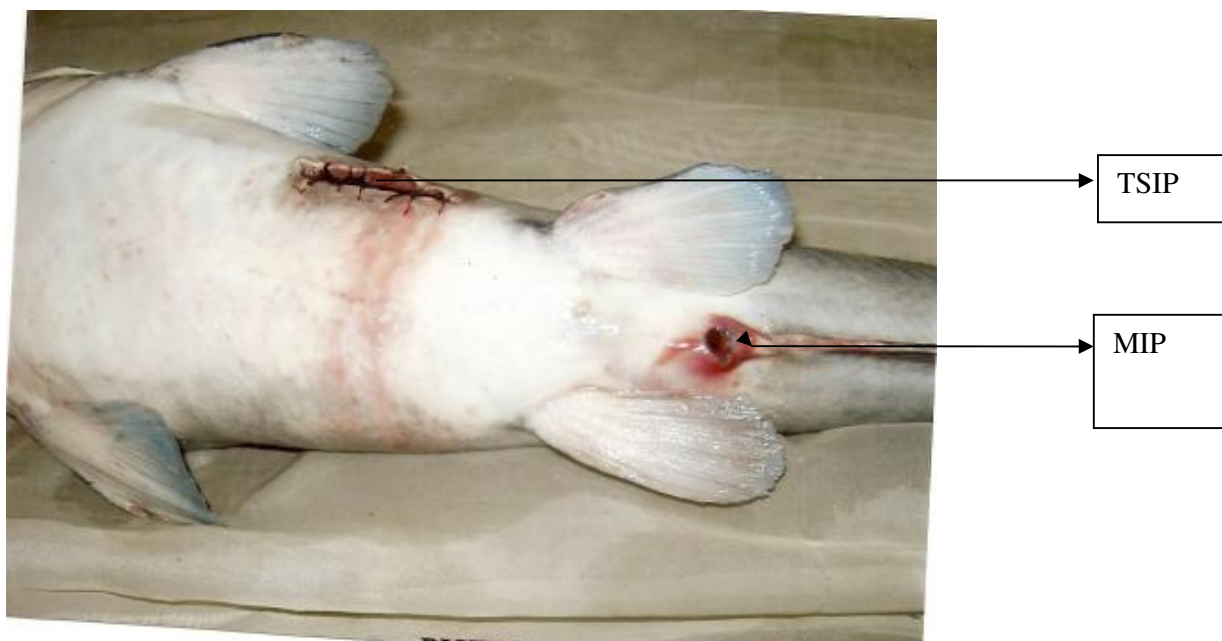
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146 Plate I: The stitched incision point on the body of dead male broodstock *H. bidorsalis* (Died
 147 after 3 days)

148 **Key:** TSIP = The Stitched Incision Point.

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Plate II: The stitched incision point on the body of dead male broodstock *H. bidorsalis* (Died after 6 days)

Key: TSIP = The Stitched Incision Point.



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158
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Plate III: The stitched incision point on the body of survived male broodstock *H. bidorsalis* (8 days after).

Key: TSIP = The Stitched Incision Point.

Discussion

The highest percentage fertility in treatment 4 (85.93^a) and hatching in treatment 3 (92.04^a) might be attributed to egg quality and viability. The eggs were dark brown and light green in colour and were not watery, an indication of good quality and viability. Treatment 1 (control) with the highest volume of milt extracted (0.86^a) was attributed to the fact that the brood stocks were sacrificed to remove testes to fertilize egg hence much milt was squeezed as compared to treatments 2, 3 and 4 where milt was extracted and obtained in little quantity. [13] made similar observation where milt volume collected through ablation in African catfish (*Clarias anguillaris*) before and after regeneration was 0.67±0.31 and 0.40 ml±0.10 respectively. The death of spent spawners at 3rd and 6th day post-surgery (plates I and II) could be due to stress and microbial infection on the incision point as a result of opening on the wound. When micro-organisms infected the wound it slows down the healing process because the body immune system (antibody) that fights against germs might be destroyed. The eight days hunger that the fish were subjected to was to facilitate the healing process because pressure on the incision point and water pollution was reduced. This however, was at variance to the work of [14] and [13]. Anaesthetic agent used on the fish was to reduce stress and ensured calmness during surgical operation as similarly reported by [13]. Out of the three incision variation lengths (Table 2) adopted, length 3.40 cm was the best that revealed the testes good enough and properly to extract milt. This method was however at variance with the method adopted by [13], milt was collected from *Clarias anguillaris* through ablation, ¾ of the testes was removed and placed in petri-dish containing saline solution (0.09 % NaCl) and preserved in refrigerator at 4^o C. Healing of the incision point (cut) occurred within 14

days post-surgery for treatments 2, 3 and 4. This corroborates the work of [13] who reported that healing of the cut occurred within 14 days in male *Clarias anguillaris*. However, this is contrary to the work of [14] who reported that cicatrization (healing) of the cut occurred within 4 weeks in male *H. longifilis* probably due to different environmental conditions and species of fish.

Incision variation length treatment 3 (3.40 cm) made on *H. birdorsalis* brood stocks had positive effect on the growth performance of the bred hatchlings (Table 3). The milt extracted were of good quality with viable eggs hence gave best growth performance in terms of body weight gain, length gain and specific growth rates as compared to other treatments. Similarly, survival rate was highest in treatment 3 (68.77 ± 224^a) as compared to other treatments. This was also attributed to good egg viability and quality milt resulted in vigour hatchlings which increases chances of high survival as similarly observed by [15]. The variation length on the abdominal region (gonad position) exposes the testes good enough to extract milt using 2 ml size syringe and needle to fertilize egg without sacrificing the male.

Conclusion

It could be concluded that incision variation length (3.40 cm) made on abdominal region (gonad position) of *H. bidorsalis* brood stocks exposed testes good enough to extract milt to fertilize egg without adverse effect on fish hence enhance fingerling production.

References

- [1] Haruna, A. B. (2003). *Aquaculture in the Tropics: Theory and Practice*. Kano Alhassan Publishers.
- [2] Food and Agricultural Organization (FAO). (2010). *Fisheries Statistics in Nigeria*. 4th ed. Abuja: FAO.
- [3] Food and Agricultural Organization/Legislation). (FAO/LEG) (2010). *Regional Compendium On Aquaculture and Inland Fisheries Legislation*. (Asian Region). Rome: FAO Development Law Service.

- [4] Mshelia, M. B., Banke, M. O., Omorinkoba, W. S., Musa, Y. M., Tafida, A. A., Richard, L. M., Ago, N. D., and Adedeji, R. B. (2007). "The Marketing and Distribution of Fish in New-Bussa Fish Market Area of Niger State." *In Proceedings of 22nd Annual Conference of the Fisheries Society of Nigeria*, 172-8.
- [5] Omeji, I. S. (2002). "Genetics for Enhancing Fisheries Production." *Nigerian Journal of Genetics* 17: 1-4.
- [6] Agbeleye, O. O., and Ipinjolu, J. K. (2001). An Assessment of the Exploitation and Management Technique of the Fisheries Resources in the Nigerian Portion of Lake Chad. *Journal of Arid Zone Fisheries* 1: 89-98.
- [7] Delince, G. A., Campbell, D., Janssen, J. A. L., and Katty, M. (1987). Fish Seed Production. In *Proceedings for Aquaculturists Course* 114.
- [8] Pandey, G. (2004). *General and Applied Itchyology*, Fishery Research. S. chad and Company Ltd. 1st Edition 2006. p.6.
- [9] Viveen, W.J.A.R., Richter, C.J.J., Van Oardt, P.G.W.J., Jassen, J.A.L. and Huisman, E.A. (1986). Practical Manual for Culture of the African Catfish, *Clarias gariepinus*. Joint Publication. Ministry for Foreign Affairs, Netherlands; Dept. of Zoology University of Utrecht, The Netherlands. 94pp.
- [10] Goudie, C.A., Scinco, B.A., Davis, K.B. and Parker, N.C. (1992). Reproductive Performance of Pigmented and Albino Female Channel Catfish Induced to Spawn with HCG or Ovaprim. *Journal of World Aquaculture Society*, 23 (2), 138-145.
- [11] Oyelese, O.A. (2006). Water Temperature a Determinant of Fertilization and Hatchability Rates in Artificially Induced Breeding of *Clarias gariepinus* (Teleostei: Clariidae). *Research Journal of Biological Sciences* 1(4), 83-87.
- [12] Jauncey, K. and Ross, B. (1982). A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture, University of Stirling U.K. 11pp.
- [13] Diyaware, M. Y., Haruna, A. B. and Abubakar, K. A. (2010). Determination of Testes Regeneration Period For African Catfish (*Clarias anguillaris*) After Milt (Semen) Collection Through Ablation. *Current Research Journal of Biological Sciences*. 2(6), 375-379.
- [14] Nguenga, D., J.J. Breine, G.G. Teugels and F. Ollevier, (1996). In: Diyaware, M. Y., Haruna, A. B. and Abubakar, K. A. (2010). Determination of Testes Regeneration Period For African Catfish (*Clarias anguillaris*) After Milt (Semen) Collection Through Ablation. *Current Research Journal of Biological Sciences*. 2(6), 375-379.
- [15] Yisa, T. A. Lamai, S. L., Tsadu, S. M. and Kolo, R. J. (2013). Induced Breeding of *Clarias gariepinus* Using Non-Conventional Method of Abdominal Incision. *International Journal of Biochemistry and Biotechnology* 2 (7), 484-489.

Table 1: Fecundity, Volume of Milt Extracted, Percentage Fertilization and Hatchability from *H. bidorsalis* for Induced Breeding Using Incision Variation Length Method (IVLM).

Treatment	IVLM (cm)	Fecundity	Vol. of Milt Extracted (ml)	Percentage Fertility (%)	Percentage Hatching (%)
1	0.00	84,924.9 ^c	0.86 ^a	68.22 ^c	72.01 ^b
2	2.50	95,266.7 ^b	0.52 ^c	54.92 ^c	63.23 ^c
3	3.40	102,101.2 ^a	0.81 ^b	72.21 ^b	92.04 ^a
4	4.00	99,686.4 ^b	0.66 ^c	85.93 ^a	69.22 ^c

Table 2: Incision Variation Lengths on Male Brood stock of *H. bidorsalis*.

Incision Length Variation (CM)				
Species type	Length I	Length II	Length III	No. of fish incised
<i>H. bidorsalis</i>	2.50	3.40	4.00	6

Table 3: Growth Performance of *H. bidorsalis* Fingerlings Bred Through Induced Breeding of Incision Variation Length Method (IVLM) for 12 Weeks.

Treatments	IVL M (cm)	MITL (cm)	MFTL (cm)	MITBW (g)	MFTBW (g)	ML G (cm)	MWG (g)	SGR
1	0.00	1.50±0.10	8.20±1.02	2.68±0.06	28.50±0.66	6.70	25.82	2.15
2	2.50	1.52±0.11	8.30±1.03	2.69±0.06	28.51±0.65	6.78	25.82	2.15

3	3.40	1.77±0.12	8.65±1.21	2.75±0.08	31.12±0.86	6.88	28.37	2.36
4	4.00	1.50±0.10	8.20±1.02	2.65±0.05	26.01±0.52	6.70	23.36	1.95

Key: IVLM= Incision Variation Length Method, MITL= Mean Initial Total Length, MFTL= Mean Final Total Length, MITBW= Mean Initial Total Body Weight, MFTBW= Mean Final Total Body Weight, MLG= Mean Length Gain, MWG= Mean Weight Gain and SGR= Specific Growth Rate.

Table 4: Grand Cumulative Mean Moortality/Survival Percentages for *H. bidorsalis* Fingerlings Bred Through Incision Variation Length Method and Reared for 12 Weeks.

Treatments	IVLM (cm)	Mortality (%)	Survival (%)
1	0.00	41.93±5.724 ^b	59.06±5.724 ^b
2	2.50	42.80±1.803 ^b	57.20±1.803 ^b
3	3.40	31.23±4.224 ^a	68.77±4.224 ^a
4	4.00	46.72±8.254 ^b	53.28±8.254 ^b

Values on the same column for each treatment carrying the same superscript did not differed significantly ($p>0.05$) from each other.