Research Article



Impact of Stocking Density on Growth Performance, Carcass Traits, and Economic Feasibility of Growing Rabbits

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Abstract | A total of sixty, 4 week-old male New Zealand White (NZW) rabbits were used to explore the impact of stocking density on the performance, carcass characteristics, blood biomarkers, and economic indices in growing rabbits. All animals were kept in wire cages (50 length x 50 width x 40 cm high) in groups of 2, 4, and 6 rabbits/cage (represented 8, 16, and 24 rabbits/m²); each stocking density was replicated 5 times. A significantly lower body weight (1748.67, 1567.58 vs. 2017.83 g) and feed intake (5040.36, 4807.47 vs. 5107.41 g) with higher feed conversion (4.22, 4.77 vs. 3.53) were observed in rabbits stocked 16 and 24 rabbits/m² compared to 8 rabbits/m², respectively (P < 0.01). No statistical variation (P > 0.05) was found in percentages of skin, full stomach, full intestine, head, and carcass parts under different stocking densities. However, a significantly lower dressing out and liver percentage (52.42 and 5.12 %, respectively) was recorded in rabbits stocked at 24 rabbits/m² than other groups (P < 0.001). Rabbits stocked at 24 rabbits/m² had the lowest values of RBCs counts, Hb, and PCV (P < 0.01). The levels of total protein, globulin, urea, and creatinine were significantly lower in the group of 24 rabbits/m² than those of 8 and 14 rabbits/m² (P < 0.05). The total and net revenue was significantly decreased and the cost-benefit ratio was increased from 0.61 to 0.77 as the number of rabbits increased from 8 to 24 rabbits/m² (P < 0.001). It can be concluded that high stocking density adversely affects rabbit performance and profitability.

Keywords | Performance, Carcass traits, Cost analysis, Rabbits

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INTRODUCTION

The rabbit production has recently gained popularity due to its high productivity, fast growth rate, efficient feed conversion ratio, and a better return on capital which are tantamount to that of broiler chicken (Gondret et al., 2005). Rabbits can be fed a diet containing up to 30% crude fiber as opposed to 10% by most poultry species and can change 20% of the protein they consume into palatable meat (Egbo et al., 2001). Rabbit meat is of high quality protein with lower calories, fat, and cholesterol levels relative to other sources of meat (Samkol and Lukefahr, 2008).

In the rabbit sub-sector, farmers must focus on optimizing kit longevity and growth efficiency; one of the best approaches to accomplish this is to establish a conductive raising environment with an appropriate stocking rate (El-Bayoumi et al., 2018). Indeed, stocking density can be defined as the number of animals per square meter in a cage, pen, or building. It is a crucial factor that can influence labor cost, investment cost, performance, and thus the profitability of rabbit production (Grace and Olorunju, 2005). The stocking densities and minimum space allowance for rabbits should often apply to the final weight achieved by animals (of a certain strain, sex, and feeding regime). The European Food and Safety Authority



(2005) suggested a base space of 625cm²/rabbit; not more than 40 kg/m² at the end of fattening to discourage rabbit behavior disorder. In particular, the ideal cage space should allow each growing rabbit to stretch along one side of the cage and sit upright at all intervals of age (Onbaslar and Onbaslar, 2007).

The economic value of stocking density derives from its negative impact on gain per animal housed and obtaining too small profit margins as overcrowding limits the space available for eating and drinking and raises aggressive animal actions (Bigler and Oester, 1996). In overcrowded cages, the nutritional and locomotive behavior of rabbits changes, which affects their carcass characteristics and meat quality (Szendrő and Dalle Zotte, 2011). In this regard, it is pertinent to explore the harmony between farm returns and a sensible degree of animal welfare regarding stocking density. Therefore, this research aimed to investigate the influence of different stocking density on growth performance, carcass characteristics, blood biomarkers, and economic efficiency of New Zealand White (NZW) rabbits.

MATERIALS AND METHODS

The experiment was conducted in compliance with the ethical standards and regulations set out by the Faculty of Veterinary Medicine's Local Laboratory Animals Care Committee, Zagazig University. The experimental practice had been approved by the institutional ethics committee (Approval No. ANWD 206).

Animals, management and experimental design

A total of sixty 4 week-old male NZW rabbits (561.7±8.3 g) obtained from a commercial rabbit farm, and were utilized in this experiment. Rabbits were randomly assigned into three groups of 10, 20, and 30 rabbits, and each group was subdivided into five replicate cages. Each replicate cage in the groups comprised of 2, 4, and 6 rabbits that correspond 8, 16, and 24 rabbits/m², respectively; where each cage measured 50 x 50 x 40 cm (length x width x high). Throughout the experiment, the lighting schedule was kept at 16 h of light and 8 h of darkness; the surrounding temperature was adjusted at 23 ±2°C and the relative humidity was about 65 ±5%. All diets were given in the pelleted form, and the basal diet was designed to fulfill the nutritional needs as per NRC Recommendations (1977; Table 1). The animals had an ad libitum excess to feed and water. The experimental procedures continued for 7 consecutive weeks (from week 4 to week 11 of age).

Performance traits

Rabbits' body weights were measured at the beginning (week 4; initial weight) and the end of the experimental

trial (week 11; final weight), and body weight gain (BWG) was estimated as the difference between final weight and initial weight. Cage feed intake (FI) was measured over this period, and the feed conversion rate (FCR) was calculated as the ratio of feed (g) to weight gain (g). The mortality rate was recorded daily throughout the experimental period. The rabbits died during the experiment were weighed, and all feed losses in each cage were gathered and weighed to ensure FI and FCR accuracy.

Table 1: Composition and chemical analyses of the basal diet (as fed basis).

| Ingredients (%) | Basal diet |
|--------------------------------|------------|
| Yellow corn | 15.3 |
| Soybean meal, 44% CP | 19.0 |
| Wheat bran | 14.0 |
| Alfalfahay, 12% CP | 32.2 |
| Barley | 12.0 |
| Corn gluten | 1.0 |
| Molasses | 3.0 |
| Dicalcium phosphate | 0.7 |
| Sodium chloride | 0.3 |
| Vit. and min. premix* | 0.3 |
| Limestone | 1.0 |
| DL-Methionine, 98% | 0.1 |
| Toxenil | 0.1 |
| Analysed nutrient content (%)‡ | |
| Crude protein | 17.4 |
| Ether extract | 2.2 |
| Crude fibre | 14.3 |
| Crude ash | 7.6 |
| Dry matter | 89.2 |
| Organic matter | 92.4 |
| Digestible energy, kcal/kg | 2508.9 |

* Vitamin and Mineral premix, provided per kg of diet: Vitamin A, 6000 IU; Vitamin D3, 1200 IU; Vitamin E, 26 IU; Vitamin K3, 1 mg; Vitamin B1, 1 mg; Vitamin B2, 3 mg; Vitamin B6, 1.5 mg; Pantothenic acid, 10 mg; Vitamin B12, 3.5 μg; Niacin, 30 mg; Folic acid, 1.25 mg; Biotin, 16 μg; Fe, 50 mg; Zn, 50 mg; Mn, 20 mg; Cu, 6 mg; I, 0.3 mg; Se, 0.03 mg; Choline chloride, 500 mg. ‡ Analysis was performed according to AOAC (2000).

CARCASS TRAITS

At the end of the study, five rabbits per group with average body weight for the corresponding group were chosen for blood sampling and carcass evaluation, earlier which they fasted for 12 h. All rabbits were weighed (representing slaughter weight, SW), euthanized by cutting the two jugulars, and then the skin, gastrointestinal tract, and distal leg segment were detached. Eviscerated carcasses (with head, liver, kidneys, thoracic cage organs, perirenal fat) were ice-cold for 24 h at 4°C and then weighed to get the chilled



carcasses weight (CCW). After that, the head, thoracic organs, liver, and kidneys were excised and the carcasses were weighed again to obtain the reference carcasses weight (RCW). The dressing out (CCW as a percentage of SW) and the proportion of head, liver, kidney, and carcass parts (fore, mid, and hind parts) to either CCW or RCW were evaluated as required.

HEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS

Blood samples (n=5/group) were taken during the slaughter process, one part was transferred to a vacutainer tube containing EDTA as an anticoagulant for hematological evaluation and the other part was transferred to a gel activator tube for serum separation (3000 rpm; 15 min; 4 °C). Red blood cells count (RBC's), white blood cells count (WBC's), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean cell hemoglobin (MCH) of the whole blood specimens were measured using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy), whereas differential leukocyte counts (lymphocytes and heterophils) were enumerated manually according to Dacie and Lewis (1991). The heterophil to lymphocyte (H: L) ratio was also determined.

Serum total protein, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea were investigated using commercial diagnostic kits (Diamond Diagnostics, Holliston, MA, USA) according to the producer's guidelines.

ECONOMIC FEASIBILITY MEASUREMENTS

A partial budgeting technique was utilized to evaluate the economic impact of stocking density as follow:

Total feed cost = Total feed intake per rabbit x cost of one kg diet

Total cost was estimated by considering feeding cost as well as the expense of experimental rabbits, veterinary services, labor and other miscellaneous expenses.

Total revenue = rabbit live body weight x price of kg live BW.

Net revenue = total revenue - total cost

Cost-benefit ratio = Total cost/total revenue

STATISTICAL ANALYSIS

Data were evaluated by one-way analysis of variance (ANOVA) using the GLM procedure in SPSS (SPSS Inc., Chicago, Illinois, USA), with individual animal or cage replicate served as the experimental unit for all statistical analyses. Shapiro-Wilk's test was applied to verify the normal distribution of data, and Levene's test to ascertain the homogeneity of variance components between experimental groups. The significance of the differences

between the mean values was estimated using Bonferroni test, where P < 0.05 was considered significant.

RESULTS AND DISCUSSION

IMPACT OF STOCKING DENSITY ON PERFORMANCE TRAITS

The impact of stocking density on the growth performance of growing rabbits is listed in Table 2. The results revealed that rabbits stocked at 16 and 24 rabbits/ m^2 had lower BW and BWG than those stocked at 8 rabbits/ m^2 (P < 0.001), with the lowest values were observed in the group of 24 rabbits/ m^2 . Also, Rabbits stocked at 16 and 24 rabbits/ m^2 exhibited lower FI (P= 0.009), and a higher FCR and mortality rate (P = 0.001 and 0.008, respectively).

Table 2: Impact of stocking density on growth performance of NZW rabbits.

| Items | Stocking | density (ra | SEM | P | |
|--------------------------|----------------------|----------------------|----------------------|-------|--------|
| | 8 | 16 | 24 | | value |
| Initial BW, g/ rabbit | 571.21 | 553.45 | 560.67 | 10.67 | 0.593 |
| Final BW, g/ rabbit | 2017.83ª | 1748.67 ^b | 1567.58° | 20.98 | <0.001 |
| BWG, g/rabbit | 1446.62a | 1195.22 ^b | 1006.91° | 16.98 | <0.001 |
| TFI, g/rabbit | 5107.41 ^a | 5040.36 ^a | 4807.47 ^b | 39.43 | 0.009 |
| FCR (feed: gain) | 3.53° | 4.22 ^b | 4.77 ^a | 0.08 | 0.001 |
| Mortality rate, % | 0.00° | 5.00^{b} | 10.00^{a} | 0.13 | 0.008 |

Means bearing different superscripts within the same row were significantly different (P < 0.05). NZW: New Zealand White rabbits; BW: body weight; TFI: total feed intake; BWG: body weight gain; FCR: feed conversion rate. SEM: standard error of the mean.

IMPACT OF STOCKING DENSITY ON CARCASS CHARACTERISTICS

Details concerning the features of the carcass are shown in Table 3. No statistical variations (P > 0.05) were found between various stocking densities concerning the percentages of skin, full stomach, full intestine, head, kidney, and carcass parts (fore, mid, and hind parts). However, a significant difference (P < 0.001) was noticed concerning dressing out and liver percentage, as the rabbits stocked at 24 rabbits/m² had a lower dressing and liver percentage as compared with those stocked at 8 and 16 rabbits/m². The SW, CCW, and RCW were significantly decreased as the stocking density increased (P < 0.001).

IMPACT OF STOCKING DENSITY ON HEMATOLOGICAL PARAMETERS

As illustrated in Table 4, the hematological values of RBCs count, Hb, and PCV % were significantly (P < 0.01) higher in rabbits stocked at 8 and 16 rabbits/m² and lower in

rabbits stocked at 24 rabbits/m². No statistical variations were detected among various stocking densities concerning MCV, MCH, WBCs, lymphocytes, heterophils and H: L ratio.

Table 3: Impact of stocking density on carcass traits of NZW rabbits.

| Items | Sto | SEM | P value | | |
|--------------------------------------|--------------------|----------------------|--------------------|-------|---------|
| | 8 | 16 | 24 | | |
| Slaughter weight (SW), g | 2085.71ª | 1816.21 ^b | 1609.07° | 25.76 | <0.001 |
| Skin, % SW | 16.75 | 17.43 | 16.89 | 1.46 | 0.123 |
| Full stomach, % SW | 4.87 | 5.28 | 5.67 | 1.04 | 0.084 |
| Full intestine, % SW | 11.54 | 12.28 | 11.93 | 1.87 | 0.231 |
| Chilled carcass weight (CCW), g | 1202.87ª | 1034.79ь | 843.56° | 20.87 | 0.003 |
| Dressing out, % | 57.67 ^a | 56.97 ^a | 52.42 ^b | 1.19 | < 0.001 |
| Head, % CCW | 9.52 | 8.87 | 9.13 | 0.88 | 0.255 |
| Liver, %CCW | 6.08^{a} | 5.93 ^a | 5.12 ^b | 0.08 | < 0.001 |
| Kidney, % CCW | 0.99 | 0.86 | 0.81 | 0.43 | 0.376 |
| Reference carcass weight (RCW), g | 997.67ª | 848.82 ^b | 639.56° | 16.76 | <0.001 |
| Perirenal fat, % RCW | 1.45 | 1.18 | 1.12 | 0.67 | 0.476 |
| Fore part. % RCW | 38.23 | 37.61 | 37.50 | 1.73 | 0.765 |
| Mid part, % RCW | 20.87 | 19.92 | 19.64 | 1.68 | 0.201 |
| Hind part, % RCW | 39.27 | 38.71 | 38.50 | 0.93 | 0.345 |

Means bearing different superscripts within the same row were significantly different (P < 0.05). NZW: New Zealand White rabbits; SEM: standard error of the mean.

Table 4: Impact of stocking density on blood hematology of NZW rabbits.

| Items | Stocking density (rabbits/m²) | | | SEM | P value |
|-------------------|-------------------------------|-------------------|--------------------|------|---------|
| | 8 | 16 | 24 | | |
| RBC, 106/μl | 5.45 ^a | 5.27 ^a | 4.33 ^b | 0.09 | 0.006 |
| Hb, g/dl | 12.45 ^a | 11.75ab | 10.60^{b} | 0.27 | 0.001 |
| PCV, % | 35.35 ^a | 34.74^{a} | 32.35 ^b | 0.36 | 0.005 |
| MCV, fl | 64.35 | 63.12 | 62.90 | 1.65 | 0.414 |
| MCH, pg | 23.91 | 23.31 | 23.35 | 0.87 | 0.724 |
| WBC, $10^3/\mu l$ | 7.50 | 8.28 | 8.74 | 0.59 | 0.238 |
| Lymphocytes, % | 69.85 | 70.13 | 70.25 | 1.09 | 0.576 |
| Heterophils, % | 36.60 | 37.51 | 37.88 | 0.65 | 0.167 |
| H: L ratio | 0.52 | 0.53 | 0.54 | 0.08 | 0.379 |

Means bearing different superscripts within the same row were significantly different (P < 0.05). NZW: New Zealand White rabbits; RBC: red blood cell count; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: Mean corpuscular hemoglobin; WBC: white blood cell count. SEM: standard error of the mean.

IMPACT OF STOCKING DENSITY ON SERUM BIOCHEMICAL PARAMETERS

Data regarding serum biochemical parameters are presented in Table 5. A significant reduction in total protein and globulin concentrations (P < 0.001) was reported as the stocking density increased. The concentrations of urea and creatinine were significantly lower (P = 0.034 and 0.017, respectively) in the group having 24 rabbits/m² compared with those of 8 and 14 rabbits/m². However, the levels of albumin, cholesterol, triglyceride, AST, and ALT were not significantly influenced by cage density (P > 0.05).

Table 5: Impact of stocking density on serum biochemical parameters of NZW rabbits.

| Items | Stockin bits/m ² | ng densit | SEM | P value | |
|---------------------------|--------------------------------|-------------------|--------------------|---------|---------|
| | 8 | 16 | 24 | | |
| Total protein, g/dl | 5.91 ^a | 5.21 ^b | 4.43° | 0.09 | < 0.001 |
| Albumin, g/dl | 3.70 | 3.53 | 3.27 | 0.29 | 0.089 |
| Globulin, g/dl | 2.21 ^a | 1.68 ^b | 1.16 ^c | 0.07 | < 0.001 |
| Total cholesterol (mg/dl) | 78.41 | 79.50 | 77.05 | 2.78 | 0.895 |
| Triglycerides, mg/dl | 126.03 | 123.50 | 121.70 | 4.34 | 0.536 |
| Urea, mg/dl | 31.86 ^a | 30.74^{a} | 26.36 ^b | 1.14 | 0.034 |
| Creatinine, mg/dl | 1.36 ^a | 1.25 ^a | 0.97^{b} | 0.05 | 0.017 |
| AST, U/1 | 25.50 | 24.11 | 24.67 | 1.31 | 0.876 |
| ALT, U/I | 81.50 | 79.21 | 81.98 | 2.56 | 0.937 |

Means bearing different superscripts within the same row were significantly different (P < 0.05). NZW: New Zealand White rabbits; AST: aspartate aminotransferase; ALT: alanine aminotransferase; SEM: standard error of the mean.

IMPACT OF STOCKING DENSITY ON ECONOMIC PARAMETERS

Economic calculations revealed that there was no significant difference among various stocking densities concerning feed cost and total costs (P > 0.05, Table 6). The best values of total and net revenue were observed in rabbits stocked at 8 rabbits/m² and the lowest values were recorded in rabbits stocked at 24 rabbits/m². The costbenefit ratio was significantly increased (P < 0.001) from 0.61 to 0.77 as the number of rabbits increased from 8 to 24 rabbits/m².

Stocking density significantly influenced the growth rate of rabbits, with those in high-density groups (16 and 24 rabbits/m²) had lower body weight and feed intake and the poorest FCR relative to the low-density group (8 rabbits/m²). The lighter body weights that were recorded for rabbits stocked at higher density may be attributable to the lower feed consumption, the lower feed utilization, and the stress that the rabbits exposed due to overcrowding. Similarly, to the current study, it has been reported that high stocking

density decreases body weight and feed consumption and increases FCR in growing rabbits (Mousa-Balabel, 2009; Baiomy, 2012; Trocino et al., 2015). Notably, Amber et al. (2018) reported that the final BW and average FI of ARPI-line rabbits decreased when the density increase from 2 to 4 and 6 rabbits per cage. On contrary, there were no significant differences among rabbits kept at a density of 1,3, and 5 rabbits/cage (Onbasilar and Onbasilar, 2007), of 4.16, 8.33, 12.5, and 16.67 rabbits/m² (Neto et al., 2007), and of 8.6, 12.9, and 17.2 rabbits/m² (Dorra et al., 2013) in final BW, BWG, and FI. Also, Volek et al. (2012) reported that stocking density (4 and 10 rabbits/m²) did not affect the FCR of Czech white rabbits.

Table 6: Impact of stocking density on economic feasibility of NZW rabbits.

| Items | Stocking density (rabbits/m²) | | | SEM | P value | |
|---|-------------------------------|-------------------|--------------------|------|------------|--|
| | 8 | 16 | 24 | | | |
| Feed cost (LE/rabbit) | 25.54 | 25.20 | 24.04 | 1.34 | 0.081 | |
| Total cost (LE/rabbit) | 55.54 | 55.20 | 54.04 | 1.33 | 0.123 | |
| Total revenue (LE/rabbit) | 90.81 ^a | 78.69b | 70.54° | 1.16 | <0.001 | |
| Net revenue (LE/rabbit) | 35.27a | 23.49b | 16.50 ^c | 0.92 | <0.001 | |
| Cost-benefit ratio | 0.61 ^c | 0.71 ^b | 0.77^{a} | 0.02 | < 0.001 | |
| Means bearing different superscripts within the same row were | | | | | | |
| significantly different ($P < 0.05$). Price of kg diet = 5 L.E; price | | | | | | |
| of kg live body weight = 45 L.E. NZW: New Zealand White | | | | | | |
| rabbits; SEM: standard error of the mean. | | | | | | |

Consistent with previous reports El-Samra et al. (2013) and Amber et al. (2018), the obtained data demonstrated that carcass traits were not significantly affected by different stocking densities except for dressing out and liver percentage, where the percentages of dressing out and liver of rabbits stocked at 24 rabbits/ m² were lower than those reared at 8 and 16 rabbits/m², likely due to the lower carcass weight of rabbits stocked at 24 rabbits/ m². However, Villalobos et al. (2008) noted that the high stocking density of 12, 18, and 24 rabbits/m² had no significant effect on carcass weight and dressing out of fattening rabbits. Yakubu and Adua (2010) noted that a stocking density of 10, 14.3, 20, and 25 rabbits/m² did not affect dressing percentage, carcass weight, and weight of visceral organs.

Our study also showed that high stocking density (24 rabbits/m²) significantly diminished estimations of RBCs, Hb, and PCV but had no impact on other blood components, in harmony with the finding of Yakubu et al. (2008) who noted that rabbits stocked at 10 and 14.3 rabbits/m² had higher PCV, Hb, and RBCs values relative to those stocked at 20 and 25 rabbits/m², though WBCs and lymphocyte counts did not differ significantly. In contrast, De la Fuente et al. (2004) stated that RBCs

and PCV values were identical in Spanish Giant rabbits housed at 8 and 12 rabbits/cage. Kalaba (2012) observed that California rabbits stocked at 4 rabbits/m² had the highest rate of MCV, MCH, and MCHC relative to those stocked at 8, 12, and 16 rabbits/m², while rabbits stocked at 16 rabbits/m² had the highest WBCs compared to those kept at 4, 8, and 12 rabbits/m².

Stocking density was found to have no impact on albumin, cholesterol, triglyceride, AST, and ALT concentrations, while the levels of total protein, globulin, urea, and creatinine were significantly decreased as the stocking density increased. Similarly, Kalaba (2012) reported that stocking density (4, 8, 12, and 16 rabbits/m²) did not change the levels of albumin and total lipid in rabbit blood plasma, but total protein, globulin, and creatinine were significantly lower in rabbits kept at higher density (16 rabbits/m²) than in those housed at lower cage densities. Onbasilar and Onbasilar (2007) noted that cholesterol and triglyceride levels in rabbit serum were not influenced by cage density (2.4, 7.1, and 11.9 rabbits/m²). In contrast, Abd El-Monem et al. (2009) concluded that there was no substantial difference in plasma total protein, globulin, and creatinine levels among rabbits housed at four stocking densities (2, 3, 4, and 5 rabbits/cage). El-Samra et al. (2013) observed that by raising the cage density, serum cholesterol, and triglyceride levels were dramatically reduced.

Economical assessment of the experimental groups revealed a substantial decline in the total and net revenue and an increase in cost-benefit ratio as the stocking density increased. In line with the current findings, Amber et al. (2018) noted that as the number of rabbits increased from 2 to 6 per cage, economic efficiency decreased from 1.17 to 0.96, and net income decreased from 18.721 to 13.204 LE. However, Verspecht et al. (2011) showed that decreasing stocking density means recalculating all costs for a decreased number of broiler rabbits, as decreasing stocking density from the standard position of 15 rabbits per m² to 10 rabbits per m² decreased added value by €22 per doe. Farm income was less and amounted to only €28.10 per doe for the reference situation of 10 rabbits per m².

CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed that increasing stocking density up to 24 rabbits/m² adversely affected economically significant traits and induced stress effects in terms of decreasing protein metabolism (total protein, globulin, and creatinine levels) and some blood disorders.

AUTHOR'S CONTRIBUTION

MAEO designed the study plan, gathered literature, and

compiled the manuscript. ME assisted in the execution of research work. FAMH provided technical assistance in writing and revising the manuscript. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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