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WELFARE ASSESSMENT OF MUSCOVY DUCKS KEPT IN TWO DIFFERENT STOCKING DENISTIES ENRICHED WITH GARLIC SUPPLEMENTATION

Basma M. Bawish , R.H. Fayed, Abeer H. Abdel Razek,

Department of Veterinary Hygiene & Management, Fac. Vet. Med., Cairo University, Egypt

ABSTRACT

This study was designed to assess the welfare of Muscovy ducks reared in two stocking densities (4 birds/m^2) (standard stocking density) and (8 birds/m^2) (high stocking density) behaviorally and immunologically if enriched with different garlic powder concentrations. A total of 180 one day old Muscovy ducklings were classified into 6 treatment groups (3 replicate/ group). groups (C, SS-G_{300}, SS+G600) raised on standard stocking density and fed on basal diet supplemented with (0, 300 and 600 g. garlic powder/ton feed), respectively while groups (HS, HS+G_{300}, HS+G600) raised on high stocking density and fed on basal diet supplemented with (0 , 300 and 600 g. garlic powder / ton feed) respectively. results indicate that enrichment of diet with (600 gm. garlic / ton ration) significantly ($p > 0.05$) decrease feather pecking, time spent feeding, increase immune organ weight, lymphocyte % and antibody titer against avian influenza vaccine in both stocking density but it increase time spent resting especially in standard stocking density however there was no significant ($p > 0.05$) difference on feeding, resting frequency and comfort behaviour percentage. It was concluded that dietary inclusion of garlic powder (600 gm. / ton feed) as feed additive had immune stimulating effects on Muscovy ducks even in high stocking density and can improve welfare of them.

Keywords: Behaviour, Garlic, immune response, Muscovy ducks, stocking density, Welfare.

INTRODUCTION

Stocking density is an important factor affecting duck production and welfare, but economic profit may come at the cost of reduced bird performance, health, and welfare if densities are excessive. (**Xie. et al (2014)** .Stocking densities differ between different strains, countries and husbandry systems. However, many producers around the world need to increase stocking densities to maximize profitability. (**Azzam and El-Gogary, 2015**). These unfavorable or stressful environmental conditions can negatively restrict birds movement so effect the access of birds to feed and water **(Estevez, et al., 1997** and **SCAHAW, 2000)** , inhibited the expression of a number of behaviours as comfort behaviour and limited

the use of specific resources so bird welfare have been impaired **(Carmichae et al., 1999**) .Stocking density also affect the bird's immune system, compromising their ability to overcome viral and bacterial infections. **(Heckert et al., 2002, Houshmand et al., 2012**), decreased the relative weights of lymphoid organs (spleen and bursa) (**Ravindran et al. 2006, Kusnadi and Rahim, 2009).**

Garlic (Allium sativum) is a one of phytobiotics that belongs to the genus Allium in the family Liliaceae. **(Eric, 2010).** Garlic supplement in poultry had been recognized for its strong stimulating effect on the immune system and this function may be attributed to the bioactive components of garlic including sulphur containing compound such as (allicin, ajoene, S-allyl cysteine.) (**Amagase and Milner 1993).** Allicin considered the main bioactive component of garlic which responsible for its biological properties (**Amagase et al., 2001, Heinrich *et al.,* 2004).** It had immune enhancing effect which include lymphocyte synthesis, cytokines release, phagocytosis and natural killer cell activity **(Tidy., et al 1990 and kyo et al., 1998) .**

Researches about the effect of garlic on immune status of ducks on different stocking density was insufficient .Therefore, the current study was conducted to assess the effects of different concentration of garlic powder supplementation on immune response of Muscovy ducks in different stocking density.

MATERIAL AND METHODS

Birds and housing

The present study was carried out at the poultry research unit at the Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University. A total number of 180 one day old Muscovy ducks of both sexes were divided into 6 treatment groups (3 replicates/group) of 10 ducks in standard stocking density groups and 20 duck in high stocking density group. The experimental ducklings were reared under the same program of management. All ducks were brooded at about 35 °C using gas heater during the 1st week of brooding period then gradually reduced 3 °C / week till reaching 25 °C at 4th week of age till the end of the study, Continuous lighting program of 23 h light and 1 h dark was maintained throughout the experimental period.

The experimental birds were reared in floor litter system (wood

shaving 10 cm depth), the experimental ducklings were vaccinated against Duck plague killed vaccine at 7 days old (0.5 ml / S.C / bird) and Avian influenza H5N1 killed vaccine at 14 days old (0.5 ml / S.C / bird) .Fresh clean water and feed were supplied ad libitum ,The feeding program consisted of A starter diet of (23.34 CP and 3020.80kcal/kg ME) that have been used from 0–4 weeks while grower diet of (19.26 CP and 3250.37 kcal/kg ME) were fed from 5 - 10 weeks (end of trials). The basal starter and grower diets were formulated to meet the nutrient requirements of Muscovy ducks according to the recommendation of the national research council **(NRC, 1994).**

Experimental design

Birds were randomly allocated into 18 symmetrical pens classified into 6 treatment groups and each group was represented by three replicates / group. The experiment carried out for 10 weeks (70 days).The 1st group fed on basal diet with standard stocking density [control (C) group], the 2nd group fed on basal diet with high stocking density (HS group), the 3rd group fed on basal diet with 300 g. garlic powder / ton feed with standard stocking density (SS-G_{300} group), the 4th group fed on basal diet with 300 g. garlic powder / ton feed with high stocking density (HS-G_{300} group), the 5th group fed on basal diet with 600 g. garlic powder / ton feed with standard stocking density (SS+G600) group), the 6th group fed on basal diet with 600 g. garlic powder / ton feed with high stocking density (HS-G_{600} group).

Parameters measured

Behavioral measurements:

Behavioral observation was continued from 2nd week of age till the end of the experiment, ten weeks. Birds in each pen were observed through two observational periods (in the morning from 8.00-11.30 a.m.) and (at afternoon from 14.00-17.30 p.m.) / 6 days. Each observation session for 3 groups X 3 replicate for 3 days, each group observed for 20 minutes /observation session which divided into 5 minute for scan sampling and 15 min for focal sampling (5 min/ each bird). Scanning sampling was used for comfort and agonistic behaviour according to **Martin and Fraser (1986**) and data was expressed as percentages. Continuous focal animal sampling **(Martin and Bateson, 1995)** was carried to obtain the frequency and duration of feeding, resting behaviour.

Immunological parameters:

Immure organ weight (spleen and bursa)

At the end of the experiment, (at 70 days old), feed was withdrawn 12 h before slaughter and 5 ducks from each replicate were randomly chosen and the weight of immune organ (spleen and bursa of Fabricious) were excised and weighed and their relative weights to live BW (%) were calculated as immune indices. **(Montgomery *et al*. 1995).**

Total leucocytic count and H/L ratio

At the end of the experiment (at 70 days old) five randomly chosen ducks from each replicate were randomly selected , slaughtered by severing of jugular vein and carotid artery and 2 ml of blood samples were placed into test tube containing anticoagulant (EDTA) for hematological studies. Total white blood cell count was carried out by using hemocytometer (**Natt and Herrick .1952).** The Differential leukocytic counts was done by preparing blood smear using standard two slide wedge procedure (**Houwen, 2000**) which fixed in methanol and stained with Wright- Giemsa Quick stain to be examined. One hundred leucocytes were counted once on each slide using a light microscope and 1000 x magnification following the method of **Davis et al. (2004).** The H/L ratios were determined by dividing the number of heterophiles by that of lymphocytes.

Humeral immune response

Blood samples were collected from five ducks from each replicate at intervals before and after 10 and 20 and 30 days of vaccination to detect the humeral immune response against Avian Influenza (AI) vaccine and to evaluate the progression of antibody titers to vaccination. Blood samples (3 ml) were obtained by sterile syringes from the jugular vein, then placed in slope position and transported in ice box to the laboratory for separation of serum. Serum was separated by centrifugation at 3000 rpm/ 10 minutes **(Swayne et al., 2008)**. The sera were stored in Eppendorf tubes at -20°C till tested. The assessment of specific antibody levels provoked by AIV vaccine were made by conventional hemagglutination- inhibition test (4 HA unit of Ag) as per **OIE standards (2008)**. The antibody titer was expressed as $\log^2$ of the reciprocal of the highest dilution.

Statistical analysis

All statistical analysis was performed using the statistical package for social science (PSPP) 22.0 for windows **(IBM CORP., NY, Armonk, 2013)**. One and two ways ANOVA were applied after exploration of data. Descriptive statistics are represented by means ± standard error (SE). The least significance difference (LSD) was used for significance among groups at significance level ($p < 0.05$).

RESULTS

Table (1): The effect of garlic powder on feeding and resting behaviour (Frequency and duration) of Muscovy ducks exposed to different stocking density

Behav,	Pattern	**(C)**	**(HS)**	**(SS+G$_{300}$)**	**(HS+G$_{300}$)**	**(SS+G600)**	**(HS+G$_{600}$)**
Feeding Behavior	**Frequency**	1.70± 0.19^{a}	2.54± 0.23^{bc}	1.80± 0.20^{a}	2.67± 0.22^{b}	1.89± 0.19^{ac}	2.85± 0.23^{b}
	duration	76.92± 1.99^{a}	55.53± 1.76^{b}	72.27± 1.74^{ac}	52.24± 1.59^{b}	67.67± 2.08^{c}	48.78± 1.66^{d}
Resting behavior	**Frequency**	2.39± 0.27^{a}	4.10± 0.21^{b}	2.21± 0.25^{a}	3.49± 0.29^{b}	2.09± 0.22^{a}	3.20± 0.28^{c}
	duration	119.72 ± 2.11^{a}	99.06± 1.78^{b}	124.18 ± 1.56^{ac}	97.90± 1.63^{b}	126.23± 2.90^{c}	102.18 ± 2.10^{b}

Different small alphabet within the same row denotes statistically difference at 0.05.
Values are expressed as means ± SE

Table (2): The effect of garlic powder on comfort and agonistic behaviour (percentage) of Muscovy ducks exposed to different stocking density

Pattern	(C)	(HS)	SS+G600)	(HS+G600)	(SS+G600)	(HS+G600)
Preening behaviour	11.00±0.33^{a}	9.41±0.25^{b}	10.88±0.25^{a}	9.11±0.20^{b}	11.28±0.28^{a}	9.90±0.23^{b}
Tail wagging behaviour	3.03±0.19^{a}	1.72±0.18^{b}	3.10±0.19^{a}	2.00±0.18^{b}	2.99±0.18^{a}	1.98±0.21^{b}
Head shaking behaviour	3.03±0.25^{a}	1.42±0.16^{b}	3.01±0.22^{a}	1.61±0.17^{bd}	3.66±0.23^{c}	2.03±0.22^{d}
Feather pecking behaviour	0.37±0.07^{a}	0.91±0.19^{a}	0.12±0.20^{b}	0.76±0.15^{a}	0.07±0.04 ^{bc}	0.44±0.09^{a}

Different small alphabet within the same row denotes statistically difference at 0.05. Values are expressed as means ± SE

Table (3): The effect of different garlic powder concentrations on immune organ weight and % of Muscovy ducks exposed to different stocking dens

Pattern	**(C)**	**(HS)**	**SS+ G600)**	**(HS+ G600)**	**(SS+ G600)**	(HS+ G600)
Spleen weight	2.44± 0.12^{ac}	2.15± 0.19^{a}	2.60± 0.28^{abc}	2.27± 0.06^{ac}	3.35± 0.51^{b}	2.93± 0.29^{bc}
Spleen %	0.076± 0.00^{ab}	0.063± 0.01^{a}	0.076± 0.00^{ab}	0.075± 0.00^{ac}	0.090± 0.01^{b}	0.085± 0.00^{bc}
Bursa weight	3.09± 0.16^{a}	2.73± 0.18^{a}	3.25± 0.19^{ac}	2.89± 0.19^{a}	4.29± 0.61^{b}	4.00± 0.36^{bc}
Bursa %	0.095± 0.01^{a}	0.091± 0.01^{a}	0.099± 0.01^{ab}	0.094± 0.00^{a}	0.116± 0.01^{bc}	0.121± 0.00^{c}

Different small alphabet within the same row denotes statistically difference at 0.05.Values are expressed as means ± SE

Table (4): The effect of garlic powder on WBCs count, Heterophil, Lymphocyte % and H/L ratio of Muscovy ducks exposed to different stocking density

Pattern	(C)	HS	SS+ G600	HS+ G600	SS+ G600	HS+ G600
TLC	105.23± 0.68^{ac}	104.58 ±0.41^{a}	105.36± 1.56^{ac}	104.75± 0.38^{a}	107.67± 0.45^{b}	106.98± 0.61^{bc}
Heterophil%	16.97± 0.49^{ac}	20.92± 0.90^{b}	15.80± 1.08^{ae}	18.68± 0.53^{c}	12.77± 0.40^{d}	14.16± 0.71^{de}
Lymphocyte %	68.85± 1.15^{ac}	65.74± 0.76^{b}	69.62± 1.43^{a}	66.95± 0.54^{bc}	74.73± 0.65^{d}	72.72± 0.86^{d}
H/L ratio	0.25± 0.01^{ac}	0.33± 0.02^{b}	0.23± 0.02^{ae}	0.28± 0.01^{c}	0.17 ±0.01^{d}	0.19± 0.01^{de}

Different small alphabet within the same row denotes statistically difference at 0.05. Values are expressed as means ± SE

Table (5): The effect of different garlic powder concentrations on antibody titer against (AIV) of Muscovy ducks exposed to different stocking density

Period	(C)	(HS)	SS+ G600	HS+ G600	SS+ G600	HS+ G600
before vaccination At 10 days of age	5.70± 0.15^{a}	5.60± 0.16^{a}	5.70± 0.15^{a}	5.70± 0.15^{a}	5.70± 0.15^{a}	5.60± 0.16^{a}
At 24 day of age	5.1± 0.23^{ac}	4.33± 0.14^{b}	5.20± 0.20^{ac}	4.67± 0.22^{ab}	5.50± 0.17^{c}	5.42± 0.15^{c}
At 34 day of age	5.25± 0.31^{ab}	4.50± 0.19^{a}	5.38± 0.26^{b}	4.92± 0.23^{ab}	6.13± 0.13^{c}	6.00± 0.12^{c}
At 44 day of age	6.00± 0.27^{ad}	5.17± 0.11^{b}	6.25± 0.25^{ad}	5.75± 0.22^{ab}	7.25± 0.31^{c}	6.58± 0.34^{cd}

Different small alphabet within the same row denotes statistically difference at 0.05. Values are expressed as means ± SE

DISCUSSION

Table (1) revealed that there was significance ($p < 0.05$) difference among groups in feeding frequencies as the least frequencies observed in groups of standard stocking density while higher frequencies observed in groups of high stocking density .On the other hand time spent feeding was significantly ($p < 0.05$) high in (C) group , ($SS+G_{300}$) group and (SS+G600) group while the lowest duration recorded in ($HS+G_{600}$) group then ($HS+G_{300}$) group and (HS) group .

These results showed that groups reared at high stocking density showed a significantly ($p < 0.05$) higher frequencies and least duration which may be due to the social facilitation at the higher density as birds were more stimulated to feeding but the competition and the difficulty of birds to spent more time on feeders decrease time spent feeding. These agreed with **Bandyopadhyay et al., (2006) and Zhao et al., (2008)** who found that the frequency of feeding were significantly higher in high stocking density. , **Zhao et al., (2008)** who found that the time spent feeding were significantly lower at 17 birds/m^2 than at 11 birds/m^2 and 14 birds/m^2. But it was noticed that garlic supplementation had no significant effect on feeding frequency however high concentration of garlic supplementation (600 gm /ton feed) decrease the time spent feeding and this may be attributed to the decrease of the feed intake by birds due to the improving effect of garlic in maximization of the nutrient absorption (**Abd El Azim et al., (2009).**

Concerning with resting behaviour in Table (1) , there was a significance ($p < 0.05$) difference in frequency of sleeping as groups of high stocking densities showed high sleeping frequency as in (HS) group, ($HS+G_{300}$) group followed by ($HS+G_{600}$) group while (C) group, ($SS+G_{300}$) group and (SS+G600) group showed lower resting frequency, These results agreed with **Hall (2001), Cornetto et al., (2002) and Febrer et al., (2006)** who reported that the frequency of resting was higher as stocking density increased. On the other hand **Zhao et al., (2008**) found that the frequency of resting was lower at 17 birds/m2 than at 11 birds/m2 and 14 birds/m^2 at 6 weeks old of broiler. However there was no significant ($p < 0.05$) effect of garlic supplementation on frequency of resting behaviour.

Regarding with time spent resting (duration), high stocking densities groups showed shorter time spent sleeping as in ($HS+G_{300}$) group, (HS) group and ($HS+G_{600}$) group however longer time spent sleeping found in standard stocking densities groups as showed in (SS+G600) group , ($SS+G_{300}$) group and (C) group , it was noticed that there was significant ($p < 0.05$) increase in time spent resting in both concentration of garlic supplementation in standard stocking density which indicate that birds more comfortable in these both groups however groups of high stocking density had less time spent resting which occurred due to increased disturbance at the higher flock size which interrupt the resting periods (**Hall ,200**1). Our results agreed with **Hall (2001**) and **Buijs et al ., (2010)** who noticed a shorter resting time at higher stocking density .On the other hand **Zhao et al., (2008**) found that the resting time was higher at high stocking density than the lower density.

Comfort behaviour

Result of comfort behaviour in Table (2) indicated that preening, tail wagging and head shaking were higher in groups of standard stocking density compared to groups of high stocking density while garlic supplementation had no significant ($p < 0.05$) effects on comfort behaviour except for head shaking which increased in group supplemented with 600 gm./ton feed . it was obvious that high density reduced comfort behaviour due to the increased disruption of birds at high stocking density **Hall (2001**) which agreed with **Albentosa and Cooper (2004**) who reported that higher density housing has been associated with birds' inability to perform comfort behaviour, including stretching and shaking behaviour. **Zhao et al., (2008**) found that the frequency of stretching, preening and shaking were significantly higher at lower stocking density.

Agonistic behaviour (feather pecking)

Results in the Table (2) indicated that (SS+G600) group recorded the lower p+-G_{600}) group **,** however high percentage of feather pecking observed in ($HS+G_{300}$) group and (HS) group . These results indicated that ducks reared at standard stocking density showed lower feather pecking and with garlic supplementation (600 gm./ton feed) these percentage lowered further and this indicate that garlic reduce stress upon birds so reduce feather pecking. These results agreed with **Bilsing et al., (1992**) who found serious injuries of ducks from feather pecking at high stocking

densities (11.6 birds/m^2) compared to ducks kept at a lower stocking density (6.3 birds/m^2) which showed no feather pecking. However it disagreed with **Ghorpade Prernam et al., (2008)** who showed that the variation among different densities in agonistic behaviour like pecking was found to be non-significant and with **(Huo, X and Na-Lampangb, P , 2016)** who showed that stocking density did not affect the feather pecking activities and aggressive behavior of Thai crossbred chickens reared at different stocking density.

Immunological parameters:

Immune organs weight and percentage

immune organs weight showed in **Table (3)** indicated that there was significant difference ($p < 0.05$) among groups in immune organs weight where higher abso;ute and relative weight of spleen and bursa observed in (SS+G600) group and (HS+G_{600}) group compared with the other groups, these results indicated that supplementation of garlic (600 gm/ton feed) increase lymphoid organ weight even in the high stocking density and this may be due to the role of the major active ingredients (allicin, ajoene, S-allyl cysteine) of garlic in enhancement of the immune response of birds **Rahmatnejad et al., (2009)** .

These results agreed with **Ibrahim et al., (2004)** who found that the weight of bursa and spleen in males and females ducks fed 3 % garlic were about 2-3 times more than the control and with **(Abd El Azim et al., 2009)** who found that feeding ducks on diet supplemented by garlic 2% & 0.2% yeast improved lymphoid organs relative weight in comparison with the other groups , **Hanieh et al., (2010)** who reported that supplementing chickens with garlic exerted enhancing effect on increasing the relative weights of the spleen and bursa of Fabricius in broiler ,but disagreed with **Kırkpınar et al., (2011)** who showed no significant differences in relative weights of spleen and bursa by supplementation of garlic oil (300 mg/kg).of feed .

Total leucocytic count, Heterophil, lymphocyte % and H / L ratio:

Total leucocytic count as in **Table (4)** was significantly ($p < 0.05$) high in (SS+G600) group and (HS+G_{600}) group compared to other groups , also there was a significant ($p < 0.05$) increase in lymphocyte (%) and decrease in heterophils (%) in (SS+G600) group and (HS+G_{600})

,while (C) group and (SS+G_{300}) group showed medium values however (HS+G_{300}) group and (HS) group showed higher Heterophils % and lower Lymphocyte % . Regarding of H/L ratio it was found that the lower values was found in (SS+G600) group and (HS+G_{600}) group while higher value observed in (HS+G_{300}) group and (HS) group. These results indicated that as birds reared at high stocking density , lower TLC , higher heterophil , lower lymphocyte % with higher H / L ratio and this attributed to the response of birds to the stressful environment which caused by increasing of stocking density , these results coincides with those of **Cravener et al., (1992**) who showed reduction in lymphocytes and increasing in heterophil % with increasing of stocking density , **Elston et al., (2000) and Spinu et al., (2003)** who found that H / L ratio increased with increasing stress level on the birds and also with (**Thaxton et al., 2005** , **Onbasilar et al ., 2008**, **Shakeri et al.,2014)**. On the other hand (**Dozier et al., 2006, Turkyilmaz ,2008 and Cengiz et al .,2015)** found that the physiological stress indicators as heterophil: lymphocyte was not affected by increasing high stocking density.

But these negative effects can be ameliorated by using garlic supplementation by higher concentration which had a great effect in improvement of immune status of ducks even in high stocking density through increases of the white blood cells, lymphocytes and immunoglobulin G (**Hanieh et al., (2010)** ,these results agreed with previous studies which showed that garlic and its contents could activate the immune function such as proliferation of lymphocyte, cytokine release, natural killer cell activity and phagocytosis **(Amagase et al., 2001; Ross et al., 2001; Patya et al., 2004; Jafari et al., 2008 and Wang et al., 2011).**

Humeral immune response

Table (5) showed antibody titer against Avian Influenza vaccine (AIV) of Muscovy ducks before and at 24, 34 and 44 d of age, it was found that there was no significant ($p < 0.05$) difference in antibody titer before vaccination among groups however at 24 day of age there was a significant ($p < 0.05$) difference among groups as the lower values observed in (HS+G_{300}) group and (HS group). On day 34 and 44 of age, the antibody titer against AIV significantly increased ($p < 0.05$) and higher values observed in (SS+G600) group and (HS+G_{600}) group while the

lowest value was observed in (HS) group, it was obvious that high stocking density decreased antibody titer against (AIV) and these results agreed with **Houshmand et al., (2012)** who mentioned that the normal stocking density of broiler resulted in higher antibody titer against Newcastle disease. While **Tong et al. (2012)** showed that no significant difference was noted in thei mmunological parameters due to different stocking density , **Azzam and El-Gogary (2015)** showed that stocking density in broiler had no effect on immunoglobulin.

However supplementation of garlic powder with higher level (600 gm/ton feed) is more beneficial in improvement of antibody response to (AIV) and this may be attributed to the bioactive components of garlic, including allicin which had immune stimulating effect. Our results agreed with **Hanieh et al., (2010)** who reported that supplementing chickens with garlic exerted enhancing effect on the humoral immune responses against Newcastle disease virus and sheep RBC (non-replicating T cell-dependent antigens) through increases of the white blood cells, lymphocytes and immunoglobulin G. and **Garba et al (2013)** who showed better immune response ($p< 0.05$) against NDV in group treated with 5g Garlic in drinking water than the control group but disagreed with (**Amera.S.Abd El-Latif et al ., 2013)** who showed that garlic essential oils (100 mg garlic oil /kg and 200 mg garlic oil /kg) failed to induce any significant effect on antibody titers in broiler.

CONCLUSION

It can be concluded that, increasing housing density affect welfare of Muscovy ducks by compromise the behaviour of birds and cause immunosuppression, which can be most easily and reliably assessed by measuring the bursa and spleen weights and or the bursa and spleen to body weight ratios. , antibody titer against (AIV) and total leucocytic count and with supplementation of garlic by high concentration (600 g. /ton) can minimize the immunosuppressive effect of high stocking density and improve welfare of birds.

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