



## Effects of Probiotic on Comfort and Body Care Behaviors of Broilers Reared at Different Stocking Densities

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### ARTICLE INFO

#### Original Research

#### Received:

04 July 2018

#### Accepted:

21 July 2018

#### Keywords:

Behavior, Broilers, Comfort, Probiotic, Stocking density

### ABSTRACT

This study was conducted to investigate the effect of probiotic on comfort and body care behaviors of broilers reared at two different densities. Two hundred and forty chicks were subdivided into four groups, two groups were reared under low stocking density (LSD); 10 bird/m<sup>2</sup> and another 2 groups were reared under high stocking density (HSD); 15 bird/m<sup>2</sup>. In each density, one group was supplemented with probiotic in the drinking water and the other was not supplemented. Normal behavior of birds was recorded throughout the growing cycle twice a week, using video camera (Sony, Japan). Then after, comfort (standing, lying and sleeping), body care (preening, dust bathing, wing/leg stretching and head scratching) and aggressive behaviors of birds were analyzed. HSD reduced comfort behavior compared to low density without probiotic group. Standing frequency was significantly increased at the 6<sup>th</sup> week of the growing cycle. In addition, interrupted lying bouts frequency was increased and the non-interrupted lying bouts (frequency and duration) were decreased significantly. Moreover, preening frequency was decreased. However, aggressive behavior was not observed in all groups. Probiotic supplementation didn't alter comfort, aggressive and body care behaviors of broilers at both densities in comparison to low density without probiotic group. However, birds stocked at high stocking density and supplemented with probiotic showed no alterations in all behavioral patterns compared to HSD group without probiotic supplementation. Data suggested that the used probiotic in this study was unable to improve comfort and body care behaviors of broilers reared at low stocking density and failed to alleviate impacts of high density stress on these behaviors.

— J. Adv. Vet. Res. (2018), 8 (3), 60-65

### Introduction

In the recent decades, intensive broiler rearing systems were increased to face the growing demand of poultry meat. Though this intensification increases production of broiler farms, behavioral alterations may occur, especially in uncontrolled environmental rearing system (Estevez, 2007). Consequently, more or less severe performance and welfare problems may be resulted (Marchewka *et al.*, 2013).

Indeed, there is a scientific debate about the appropriate and stressful stocking densities. Thomas *et al.* (2004) found no difference in feed intake of broilers reared at 10, 15 and 20 bird/m<sup>2</sup> densities. In addition, Turkyilmaz (2008) reported no alteration in feed intake of birds 15, 20, 25 bird/m<sup>2</sup> rearing densities. Moreover, feed consumption was not significantly influenced by increasing stocking density from 11 to 14 or 17 bird/m<sup>2</sup> (Zhao *et al.*, 2009).

Broilers spend most of their time lying down (Bessei, 1992). This time is controlled by total space availability (Mur-

phy and Preston, 1988). Furthermore, as the birds grow and get heavier spending more time lying and visit the feeders less often (Savory and Lariviere, 2000; Weeks *et al.*, 2000).

Comfort behavior is closely linked with poultry meat carcass quality (Cornetto *et al.*, 2002). Therefore, fragmentation of comfort behavior leads to less sleeping time, which may retard growth and performance of birds (Malleau *et al.*, 2007). In addition, chickens require space to accommodate normal body functions and also for maintaining normal behaviour such as body care. Moreover, adequate floor space is required for performance of birds' social behavior (Albentosa and Cooper, 2004). Reduction of social behavior performance may increase aggression between birds. Feather pecking and aggressive encounters are considered as important welfare and management concern in intensive poultry farms (Marchewka *et al.*, 2013). Hence, decreasing floor space restricts social interactions and body care behavior that may increase incidence of welfare problems occurrence.

There is a close correlation between brains and bowels that allows the individual's gut bacteria to steer their behavior (Yong, 2011). Furthermore, probiotic bacteria can reduce stress and depression related behaviors (Sudo, 2006; Messaoudi *et al.*, 2011). Therefore, our goal was to investigate effects of the

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used probiotic (Protexin®) on comfort and body care behaviors of broilers reared at two different stocking densities (10 and 15 birds/m<sup>2</sup>).

## Materials and methods

### Experimental design

This study was conducted in the poultry house belonging to Department of Animal and Poultry Management and Wealth Development, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Two hundred and forty unsexed one-day old (Cobb type breed) chicks were purchased from a commercial hatchery and brooded at 33°C using electric heaters for the first week of age. Then, they were distributed into four groups at the end of the first week (two low density groups and other two high density groups). In each density, one group supplemented with probiotic and the other did not supplemented with probiotic. Ninety six birds were used in low stocking density (LSD) groups (10 bird/m<sup>2</sup>); 48 chicks in each group; three replicates for each (16 bird/ replicate). For high stocking density (HSD) groups (15 bird/m<sup>2</sup>), 144 birds were divided into two equal groups; three replicates (24 bird / replicate) in each.

The study was approved by Institutional animal care and use committee of Beni-Suef University (BSU-IACUC), Egypt.

### Birds accommodation and management

All chicks were reared in 12 floor pens (1m × 1.6 m), a new wood shaving litter material was used. The ventilation was maintained using windows, fans and exhausting fans. Heating during brooding was maintained by the electric heaters, with a decrease in the temperature 2°C each week. Lighting program was set as continuous lighting for the first week, 23 hrs light and 1 hr dark till the end of the experiment by well-distributed red bulbs. All birds were reared under the same environmental conditions. Feed and water were provided ad libitum. The birds were fed using a two-phase broiler-feeding regime; a starter (23% protein) crumble for the first 21 days, followed by a grower pellet (21% protein) until the end of the study at day 42.

### Protexin® probiotic supplementation

Protexin® probiotic (Enterococcus faecium 2 x 10<sup>9</sup> CFU/g (2 x 10<sup>12</sup> CFU/kg)) was purchased from probiotic International Smorest, UK. It was added to the drinking water of birds starting from the second week according to the manufacturer recommendation.

### Measurement of behavior

The behaviour of chicks was recorded twice a week, in the morning (9:00 – 12:00 am) for 5 consecutive weeks. Behavior of each replicate was recorded using video camera (Sony, Japan) for 20 minutes. Then after, behavior of birds was analyzed using scan method of observation that adopted according to Maria *et al.* (2004). Comfort behavior was analyzed including standing lying and sleeping. Lying bouts were classified into non-interrupted lying bouts (NILBs) and interrupted lying bouts (ILBs). The later was analyzed as ILB1 (lying bouts interrupted by other birds, ILB2 (lying bouts interrupted by standing) and ILB3 (lying bouts interrupted by adjusting the bird's position)

Comfort behavior analysis was expressed as weekly frequency of standing, interrupted and non-interrupted lying bouts weekly frequency, weekly non-interrupted lying total duration and weekly frequency of sleeping behavioral pattern. In addition, frequency of body care (preening, dust bathing, wing or leg stretching), and behavioral patterns throughout the growing cycle was analyzed:

### Statistical analysis

Data was analyzed by one-way ANOVA test (Dunnet test) using SPSS (Statistical Package for Social Science, 2011). Results considered significant at P<0.05. Results of the four groups at the same week of the growing cycle were compared statistically while, the differences of data within groups were not analyzed.

## Results

By comparing results of the 4 groups using One Way ANOVA test, it was found that comfort and body care behaviors were significantly impaired in birds reared at high group size with and without probiotic supplementation (group 3 and 4. Standing frequency was significantly (P<0.05) increased at the 6<sup>th</sup> week of the growing cycle (Table 1).

Though weekly frequency of total lying bouts was not significantly altered, ILBS frequency was increased in expanse of non-ILBs frequency (Table 2). There was a significant increase (p<0.05) in weekly frequency of ILB2 and ILB3 at the 6<sup>th</sup> week and at the 5<sup>th</sup> and 6<sup>th</sup> weeks of the growing cycle, respectively (Table 2). On contrary, weekly frequency of non-ILBs of group 3 and 4 was decreased significantly (P<0.05) at 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> week of the growing cycle (Table 2). In addition, non-ILB duration/sec was significantly shortened at the 2<sup>nd</sup> (p<0.05), 3<sup>rd</sup> (P<0.05), 4<sup>th</sup> (P<0.05), 5<sup>th</sup> (p<0.01) and 6<sup>th</sup> (P<0.05) weeks of the growing cycle (Table 3). Moreover, sleeping frequency

Table 1. Effect of probiotic supplementation on weekly frequency of standing behaviors at different stocking densities.

Groups	Weekly Standing frequency "SF"				
	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
1	9.67±0.88 <sup>a</sup>	8.00±1.55 <sup>a</sup>	8.00±2.00 <sup>a</sup>	6.00±0.58 <sup>a</sup>	6.00±1.00 <sup>b</sup>
2	9.33±1.20 <sup>a</sup>	8.00±1.00 <sup>a</sup>	7.33±1.45 <sup>a</sup>	5.67±0.67 <sup>a</sup>	5.67±0.67 <sup>b</sup>
3	12.00±0.58 <sup>a</sup>	8.67±1.33 <sup>a</sup>	9.33±0.88 <sup>a</sup>	8.33±0.88 <sup>a</sup>	9.67±0.33 <sup>a</sup>
4	12.33±2.19 <sup>a</sup>	10.00±2.65 <sup>a</sup>	7.67±0.67 <sup>a</sup>	8.67±0.67 <sup>a</sup>	10.00±0.58 <sup>a</sup>

Results were expressed as Means ± Standard Error (S.E.).

<sup>a,b</sup>Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m<sup>2</sup> rearing density without probiotic supplementation

2= Gp2=10 bird/m<sup>2</sup> density with probiotic supplementation

3= Gp3=15 bird/m<sup>2</sup> rearing density without probiotic supplementation

4= Gp4=15 bird/m<sup>2</sup> rearing density with probiotic supplementation

showed no significant decrease in both high group size (with and without probiotic supplementation) (Table 4). Furthermore, frequency of preening behavior throughout the growing cycle was reduced significantly ( $P < 0.05$ ) in birds stocked at high density (Table 5). By comparison of low stocking density groups (1 and 2), it was clear that probiotic supplementa-

tion did not significantly improve comfort and body care behaviors of broilers. Similar results were noticed when data of high stocking density groups (3 and 4), In all groups, aggression and abnormal behaviors were not observed between birds.

Table 2. Effect of probiotic supplementation on the weekly frequency of different lying bouts at different stocking densities.

Groups	Total LBs	Non-interrupted LBs	Interrupted LBs (ILBs)			
			ILB1	ILB2	ILB3	
Week						
1	2 <sup>nd</sup>	25.00±2.52 <sup>a</sup>	18.67±1.20 <sup>a</sup>	2.33±0.89 <sup>a</sup>	ND	4.00±0.58 <sup>a</sup>
	3 <sup>rd</sup>	22.33±2.03 <sup>a</sup>	15.00±1.73 <sup>a</sup>	2.33±0.67 <sup>a</sup>	0.67±0.67 <sup>a</sup>	4.33±0.33 <sup>a</sup>
	4 <sup>th</sup>	18.00±1.00 <sup>a</sup>	11.00±0.57 <sup>a</sup>	2.00±0.66 <sup>a</sup>	1.00±0.58 <sup>a</sup>	4.00±1.15 <sup>a</sup>
	5 <sup>th</sup>	15.67±1.45 <sup>a</sup>	9.33±0.88 <sup>a</sup>	2.00±1.15 <sup>a</sup>	1.33±0.67 <sup>a</sup>	3.00±1.15 <sup>b</sup>
	6 <sup>th</sup>	16.00±1.15 <sup>a</sup>	8.67±0.88 <sup>a</sup>	1.67±1.20 <sup>a</sup>	0.33±0.33 <sup>b</sup>	5.33±0.88 <sup>b</sup>
2	2 <sup>nd</sup>	23.33±0.67 <sup>a</sup>	17.33±1.45 <sup>a</sup>	3.00±0.87 <sup>a</sup>	ND	3.00±1.00 <sup>a</sup>
	3 <sup>rd</sup>	22.00±2.64 <sup>a</sup>	15.67±2.33 <sup>a</sup>	2.33±0.33 <sup>a</sup>	1.00±0.58 <sup>a</sup>	3.00 ± 0.58 <sup>a</sup>
	4 <sup>th</sup>	16.33±1.20 <sup>a</sup>	11.33±1.86 <sup>a</sup>	2.00±0.57 <sup>a</sup>	0.67±0.67 <sup>a</sup>	2.33±0.33 <sup>a</sup>
	5 <sup>th</sup>	17.33±0.67 <sup>a</sup>	11.00±1.15 <sup>a</sup>	3.00±0.58 <sup>a</sup>	1.33±0.33 <sup>a</sup>	2.00±1.00 <sup>b</sup>
	6 <sup>th</sup>	16.33±1.76 <sup>a</sup>	10.00±1.15 <sup>a</sup>	2.33±0.88 <sup>a</sup>	0.67±0.33 <sup>b</sup>	3.33±1.33 <sup>b</sup>
3	2 <sup>nd</sup>	21.67±1.21 <sup>a</sup>	11.00±0.58 <sup>b</sup>	5.33±1.67 <sup>a</sup>	1.00±0.57 <sup>a</sup>	4.33±0.88 <sup>a</sup>
	3 <sup>rd</sup>	18.33±0.88 <sup>a</sup>	7.00±1.00 <sup>b</sup>	5.67±1.76 <sup>a</sup>	1.00±0.00 <sup>a</sup>	4.67 ± 0.88 <sup>a</sup>
	4 <sup>th</sup>	19.00±1.53 <sup>a</sup>	5.67±0.67 <sup>b</sup>	6.00±0.88 <sup>a</sup>	1.33±0.33 <sup>a</sup>	6.67±1.67 <sup>a</sup>
	5 <sup>th</sup>	20.33±1.20 <sup>a</sup>	4.67±0.88 <sup>b</sup>	5.67±0.33 <sup>a</sup>	1.00±1.00 <sup>a</sup>	9.00±1.00 <sup>a</sup>
	6 <sup>th</sup>	24.00±2.89 <sup>a</sup>	4.00±1.00 <sup>b</sup>	4.00±0.58 <sup>a</sup>	4.00±0.57 <sup>a</sup>	12.00±1.52 <sup>a</sup>
4	2 <sup>nd</sup>	22.67±3.38 <sup>a</sup>	11.67±1.45 <sup>b</sup>	6.00±1.00 <sup>a</sup>	1.33±0.89 <sup>a</sup>	3.67±0.67 <sup>a</sup>
	3 <sup>rd</sup>	16.33±2.91 <sup>a</sup>	6.33±1.20 <sup>b</sup>	4.33±1.33 <sup>a</sup>	1.33±0.33 <sup>a</sup>	4.33±0.66 <sup>a</sup>
	4 <sup>th</sup>	19.33±2.33 <sup>a</sup>	5.33±0.88 <sup>b</sup>	5.33±1.33 <sup>a</sup>	1.67±0.33 <sup>a</sup>	7.67±1.45 <sup>a</sup>
	5 <sup>th</sup>	19.00±3.06 <sup>a</sup>	4.67±0.33 <sup>b</sup>	4.33±1.45 <sup>a</sup>	1.33±0.88 <sup>a</sup>	8.67±1.20 <sup>a</sup>
	6 <sup>th</sup>	23.33±1.86 <sup>a</sup>	3.33±0.88 <sup>b</sup>	4.67±1.20 <sup>a</sup>	4.33±0.88 <sup>a</sup>	11.00±0.58 <sup>a</sup>

Results were expressed as Means ± Standard Error (S.E.).

<sup>a,b</sup> Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m<sup>2</sup> rearing density without probiotic supplementation (control)

2=Gp2=10 bird/m<sup>2</sup> density with probiotic supplementation

3= Gp3=15 bird/m<sup>2</sup> rearing density without probiotic supplementation

4= Gp4=15 bird/m<sup>2</sup> rearing density with probiotic supplementation

LBs: Lying bouts; NILB: non-interrupted lying bouts; ILBs: Interrupted lying bouts; ILB1: caused by other birds

ILB2: caused by standing; ILB3: caused by adjusting the bird's position; ND: Not detected

Table 3. Effect of probiotic supplementation on the weekly non-interrupted lying total duration at different stocking densities

Groups	Weekly total duration of NIL (sec)				
	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
1	3218.33 ±238.30 <sup>a</sup>	2850.00 ±350.19 <sup>a</sup>	2200.00 ± 136.14 <sup>a</sup>	1988.33 ±149.51 <sup>a</sup>	1771.67 ±337.30 <sup>a</sup>
2	3100.00 ±228.11 <sup>a</sup>	2920.00 ±472.90 <sup>a</sup>	2283.33 ±536.45 <sup>a</sup>	2340.00 ±287.46 <sup>a</sup>	2045.00 ±253.20 <sup>a</sup>
3	1870.00 ±232.45 <sup>b</sup>	1210.00 ±101.49 <sup>b</sup>	996.67 ±131.19 <sup>b</sup>	850.00 ±132.29 <sup>b</sup>	640.00 ±185.20 <sup>b</sup>
4	1996.67 ±265.60 <sup>b</sup>	1112.67 ±212.85 <sup>b</sup>	990.00 ±150.99 <sup>b</sup>	843.33 ±33.33 <sup>b</sup>	530.00 ±177.76 <sup>b</sup>

Results were expressed as Means ± Standard Error (S.E.).

<sup>a,b</sup> Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m<sup>2</sup> rearing density without probiotic supplementation

2=Gp2=10 bird/m<sup>2</sup> density with probiotic supplementation

3= Gp3=15 bird/m<sup>2</sup> rearing density without probiotic supplementation

4= Gp4=15 bird/m<sup>2</sup> rearing density with probiotic supplementation

NIL= non-interrupted lying

Table 4. Effect of probiotic supplementation on the weekly frequency of sleeping behavioral pattern at different stocking densities.

		Weekly sleeping frequency				
		2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
Groups	1	24.33±4.37 <sup>a</sup>	12.67±2.33 <sup>a</sup>	12.00±1.73 <sup>a</sup>	11.33±1.86 <sup>a</sup>	15.67±6.23 <sup>a</sup>
	2	23.33±6.01 <sup>a</sup>	14.00±2.52 <sup>a</sup>	13.33±1.67 <sup>a</sup>	12.33±0.33 <sup>a</sup>	11.00±2.08 <sup>a</sup>
	3	20.00±1.15 <sup>a</sup>	11.33±2.34 <sup>a</sup>	8.00±1.02 <sup>a</sup>	9.67±1.84 <sup>a</sup>	4.00±1.00 <sup>a</sup>
	4	17.67±1.45 <sup>a</sup>	10.67±3.48 <sup>a</sup>	7.67±1.45 <sup>a</sup>	11.00±2.52 <sup>a</sup>	4.33±1.20 <sup>a</sup>

Results were expressed as Means ± Standard Error (S.E.)

<sup>a,b</sup> Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m<sup>2</sup> rearing density without probiotic supplementation

2=Gp2=10 bird/m<sup>2</sup> density with probiotic supplementation

3= Gp3=15 bird/m<sup>2</sup> rearing density without probiotic supplementation

4= Gp4=15 bird/m<sup>2</sup> rearing density with probiotic supplementation

Table 5. Effect of probiotic supplementation on the frequency of body care behavioral patterns throughout the growing cycle at different stocking densities.

		Body care behavioural patterns			
		Preening	Dust bathing	Wing or leg stretching	Head scratching
Groups	1	16.133±1.43 <sup>a</sup>	0.80±0.12 <sup>a</sup>	2.53±0.18 <sup>a</sup>	2.60±0.31 <sup>a</sup>
	2	15.40±0.75 <sup>a</sup>	0.67±0.18 <sup>a</sup>	2.40±0.31 <sup>a</sup>	2.20±0.50 <sup>a</sup>
	3	10.13±1.25 <sup>b</sup>	0.33±0.13 <sup>a</sup>	1.47±0.07 <sup>a</sup>	1.13±0.18 <sup>a</sup>
	4	10.33±0.98 <sup>b</sup>	0.47±0.13 <sup>a</sup>	1.80±0.35 <sup>a</sup>	1.20±0.40 <sup>a</sup>

Results were expressed as Means ± Standard Error (S.E.)

<sup>a,b</sup> Superscripts within columns indicate significant difference

1= Gp1= 10 bird/m<sup>2</sup> rearing density without probiotic supplementation

2=Gp2=10 bird/m<sup>2</sup> density with probiotic supplementation

3= Gp3=15 bird/m<sup>2</sup> rearing density without probiotic supplementation

4= Gp4=15 bird/m<sup>2</sup> rearing density with probiotic supplementation

## Discussion

The results of our study revealed the impacts of increasing group size of broiler on comfort and body care behaviors in addition to the role of probiotic supplementation in alleviation of these effects.

Results of this study indicated that HSD induced significant increase in standing frequency only at last week of the growing cycle. This was agreeable with Buijs *et al.* (2010, 2011), while, Andrews *et al.* (1997) found that standing duration wasn't affected by stocking density.

The obtained results in this study indicated the marked disturbance in lying behavior of birds reared at HSD at the end of the growing cycle. Similarly, Lewis and Hurnik (1990) and Buijs *et al.* (2012) observed shorter lying bouts of birds tucked at high density compared to control. On the other hand, Andrews *et al.* (1997) stated that total time spent lying was not significantly affected by stocking density and Martrenchar *et al.* (1997) mentioned that the duration of LB (lying bouts) did not relate to stocking density.

It is worthy noted that, the effect of HSD stress on comfort behavior was prominent at the end of the growing cycle. This might be attributed to increase size of the birds and con-

sequently floor space relatively decreased by age.

The recorded increase in standing frequency resulted in the increase of ILBs frequency because standing of birds during lying is an interruption of the lying bouts. In addition, increase of ILB frequency suggested that birds were restless rather than resting (Murphy and Preston, 1988). There are three possible explanations of increasing birds' ILB frequency. The first is the disturbances caused by other birds (Lewis and Hurnik, 1990). This was confirmed by Martrenchar *et al.* (1997), who declared that when a bird walks to a feeder or drinker in an overcrowded shed, it is likely to pump into other, remove away or move their heads away from the interference whilst they remain sitting. The second explanation is that the birds may frequently stand as cooling strategy to alleviate heat stress (Murphy and Preston, 1988; Lewis and Hurnik, 1990; Zulkifli and Sti nor Azah, 2004). This is because increased contact with neighboring birds at HSD limited the area available for heat transfer to 33% of a bird's total surface (Murphy and Preston, 1988). Thus, birds tend to adjust their sitting posture (Buijs *et al.*, 2012). The third suggested explanation is the high litter content of ammonia that may cause irritation in respiratory tract of the birds, hence they stand as an attempt to decrease its inhalation.

This restless behaviour (interrupted lying), which was a reflection of cooling strategy or due to pumping into other birds on the floor may have implications on bird welfare and production efficiency. For example, increases the risk of carcass scratching and bruising (Murphy and Preston, 1988).

The observed fragmentation of resting behavior resulted in corresponding decrease in sleeping frequency was agreeable with Malleau *et al.* (2007). However, Andrews *et al.* (1997); Bessei (1992) and Sanotra *et al.* (2002) found that birds stocked at HSD spent more time dozing and sleeping than those at lower density.

The reported reduction of frequency of body care behavior especially preening is similar to the result recorded by Andrews *et al.* (1997). This was because chickens require adequate space to perform normal grooming (Albentosa and Cooper, 2004). This space was diminished by increasing stocking density, which consequently reduces performance of preening behavior.

Moreover, aggressive behavior was not recorded in this study neither in high nor low stocking densities. Similar results were recorded by Murphy and Preston (1988) and Preston and Murphy (1989). On the other hand, many studies reported aggression in broiler reared at HSD (Puron *et al.*, 1995; Feddes *et al.*, 2002). Mean a while, Keeling and Duncan (1991) reported that aggressiveness is relatively higher in small flocks than in large flocks, as birds adopt strategies to avoid negative social interactions.

In the present study, protexin® supplementation didn't alter comfort, aggressive and body care behaviors of broilers at low density. In addition, birds stocked at high stocking density and supplemented with Protexin® showed no alterations in all behavioral patterns compared to HSD group. It means that, the used probiotic was not effective in mitigating stressful effect of HSD on comfort and body care behaviors broilers.

Stressful effect of HSD on the bird's behaviors may be attributed to some factors such as the competition of birds on feed and water (Rashidi *et al.*, 2010), increasing litter content of moisture and ammonia (Dawkins *et al.*, 2004) and the increased air carbon dioxide (Yardimci and Kenar, 2008). These factors may be prevented or corrected by probiotic supplementation (Endo and Nakano, 1999; Zhang and Kim, 2013). This was unlike our study results. Hence, the used probiotic (Protexin®) could not correct or prevent these stressful effects of HSD on broilers.

## Conclusion

Based on our results it may be concluded that, Protexin® was unable to improve comfort and body care behaviors of broilers reared at low stocking density and failed to alleviate impacts of high density stress on these behaviors.

## Acknowledgement

We acknowledge Projects Funding and Granting unit belonging to the Scientific Research Developing Unit in Beni-Suef University, Egypt for funding this study.

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