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Research Article
Impact of Chinese Royal Jelly on Performance, Behaviour and Some Blood Parameters in Broilers Reared under High Stocking Density

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Abstract

Objective: This experiment was conducted to assess the use of Chinese Royal Jelly (RJ) over a 6 weeks period on performance, behaviour and blood parameters of broilers. **Methodology:** Approximalty,180 one-day-old Ross broiler chicks were randomly assigned to six treatment groups as follows; Group 1: Control (LSD; 8 birds, per meter square and no supplementation), Group 2: High stocking density (HSD; 16 birds per meter square and no supplementation), Group 3: LSD-RJ 250 (8 birds per meter square and 250 mg kg^{-1}), Group 4: LSD-RJ 500 (8 birds per meter square and 500 mg kg^{-1} b.wt.), Group 5: HSD-RJ 250 (16 birds per meter square and 250 mg kg^{-1} b.wt.) and Group 6: HSD-RJ 500 (16 birds per meter square and 500 mg kg^{-1}) RJ given orally. Scan sampling for comparative behavior in all groups was carried out at 2 min intervals during 2-20 min periods for 5 days each week. **Results:** The highest Feed Intake (FI), Feed Conversion Rate (FCR) and Body Weight Gain (BWG) were recorded in the LSD group ($p<0.05$) fed on diet supplemented with Chinese Royal Jelly (RJ) compared to other treatments during the experimental period. Also, FI, FCR and BWG were significant ($p<0.05$) improved in HSD-RJ 250 and HSD-RJ 500 compared to other groups. It was found that a significant higher proportion of chicks in RJ supplemented groups were engaged in feeding, resting, walking, standing, foraging and preening behaviour however, drinking behaviour was not significant influenced ($p>0.05$) by RJ supplementation. Chicks fed 500 mg of RJ were found to have the highest values of the heterophils and lymphocytes. Serum total protein, albumen and globulin concentrations were significantly ($p<0.05$) boosted in the RJ groups compared to the controls. **Conclusion:** The study was completed concluding that Chinese royal jelly supplementation can alleviate the deleterious effect of high stocking density in Ross broiler chicks, improved behaviour, performance and blood parameters in the examined broiler chicks under high stocking density.

Key words: Broilers, royal jelly, stocking density, body weight, feed intake, feed conversion rate, behaviour, total protein, heterophils, lymphocytes

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In broiler production systems, stocking density (or floor space allowance per bird) is believed to have major implications on bird performance and has recently been identified as an important welfare concern in the broiler industry[1]. A number of studies have investigated the effect of various stocking densities on behavioural traits in broiler chickens as reviewed by Ekstrand and Carpenter[2]. It is known that increasing the stocking density leads to changes in behaviour in both commercial[3] and experimental conditions[4]. In general, increasing the number of birds per unit area was found to increase the incidence of agonistic behaviour and reduce the amount of time spent lying (resting) behaviour. In recent years, there has been renewed interest in the investigation of bee products (honey, pollen, Royal Jelly (RJ), bee venom and propolis) for their numerous functional, biological and pharmaceutical beneficial effects[5,6]. The RJ is a honey bee (*Apis mellifera* L.) secretion component for bee egg, larvae and adult queen nourishment. This secretion is extremely rich in nutrients and has a yellowish-white, creamy liquid appearance and an acidic pH. It is secreted by the hypo-pharyngeal glands of nurse bees. The overall composition of RJ is 60-70% water, 12-16% crude protein, 10-16% total sugar, 3-6% lipids, vitamins and mineral salts. The composition varies according to the source of the exudates, climate and some environmental conditions. The RJ contains many bioactive ingredients. The main bioactive material is 10-hydroxy-2-decenoic (HDA, RJ acid) an unsaturated acid that is only found in RJ in nature. Numerous HDA effects including antibacterial, antifungal, antiviral[7,8] and immunoactivating[9] effects were reported. The RJ contains major proteins with high levels of essential amino acids and peptides[10], possessing immunomodulating[11] and antioxidant properties[9]. Phenols and polyphenols in major protein structures are responsible for significant antioxidant activity[7]. The RJ is rich in vitamins especially, water-soluble vitamins (vitamins B and C) and minerals such as potassium, calcium, magnesium, iron, zinc, sulfur and copper[12]. The continuously increasing demand for protein for human consumption has prompted some producers and researchers to search for new studies in animal production and husbandry practices in order to improve production efficiency. Cage density is an important environmental factor affecting production levels and quality in poultry meat[13]. The goal of poultry producers is to achieve a balance between production efficiency and bird welfare. Increasing the bird number per unit of space (density) reduces housing, equipment and labor costs. It is well documented that chickens housed at high density grow more slowly and have higher mortality and lower production quality[14]. On the other hand, the RJ has an antioxidant effect that counteracts the lipid per oxidation caused by free radicals under different stress conditions[7]. The purpose of this study was to examine the potential protective effects of RJ for alleviating the metabolic, hormonal and blood parameters changes resulted from high stocking density.

MATERIALS AND METHODS

Birds and experimental design: The experiment was conducted in accordance with animal welfare. A total of 180 one-day-old unsexed broiler chicks (Ross 308) were randomly divided into six equal groups according to their initial body weights. Each group contained three replicates of 10 chicks obtained from a commercial company were used for this study. The chicks were grown in a separate floor pens with wood shavings and lighting program of 23 h lightning and 1 h darkness day^{-1} constant light. Food and fresh water were provided *ad libitum* using a two-phase broiler feeding regime. This consisted of a commercial starter crumbles for the first 21 days, followed by a commercial grower/finisher pellets until the conclusion of the study at day 42. The ingredients and chemical composition of the basal diet and calculated energy uptake per kilogram diet are presented in Table 1. The

Table 1: Ingredients and chemical composition of the basal diet (g kg^{-1}) and energy uptake (kilogram per diet)

Ingredients (g kg^{-1})	Starter (day 21)	Grower (days 22-42)
Corn	50.5	60.05
Fish meal	3.5	3
SBM	36.75	29
Sunflower oil	6	4.7
Dicalcium phosphate	1.5	1.5
Ground lime stone	1	1
Salt	0.3	0.3
Lysine	0.1	0.1
Methionine	0.1	0.1
Premix	0.25	0.25
Calculated energy uptake kilogram per diet	Starter	Grower
Energy (Kcal kg^{-1})	3202.465	3208.048
Crude protein (%)	22.977	20.0363

SBM: Soya bean meal

temperature was maintained at 34°C on day 1 and was gradually reduced to 22°C by day 21. The relative humidity was maintained at 40-65%. The experiment was performed during winter and open windows were used to achieve cross-ventilation. All experimental birds were reared as per the recommendations of the National Research of the US[15] in a temperature-controlled room at 24°C. Each broiler chicken was vaccinated against Newcastle Disease Virus (NDV) via drinking water at 6, 14, 21 and 32 days of age and against Infectious Bursal Disease (IBD) at 10, 18 and 25 days of age. The normal stocking density was (10 birds per meter square) and the high stocking density was (15.4 birds per meter square) according to Sorensen *et al*.[16]. The stocking densities were coded Low Stocking Density (LSD) and High Stocking Density (HSD) and contained a floor space allowance per meter squar of 8 and 16 birds, respectively. The experimental groups were reared for 1-42 days. Groups were arranged and fed as follows: Group 1: Control group; Low stocking density (LSD; 8 birds per meter square and no supplementation), Group 2: High stocking density (HSD; 16 birds per meter square and no supplementation), Group 3: LSD-RJ 250 (LSD; 8 birds per meter s quare and 2 50 mg kg^{-1} b.wt.), Group 4: LSD-RJ 500 (LSD; 8 birds per meter square and 500 mg kg^{-1} b.wt.), Group 5: HSD-RJ 250 (HSD; 16 birds per meter square and 250 mg kg^{-1} b.wt.) and Group 6: HSD-RJ 500 (HSD; 16 birds per meter square and 500 mg kg^{-1} b.wt.).

Sample preparation and identification: Chinese Royal Jelly (RJ) was obtained from a commercial firm in China, dissolved in distilled water and kept frozen at -20°C until used. Chemical composition of Chinese Royal Jelly (RJ) was assessed by Gas Chromatography/Mass Spectrometry (GC/MS). The GC/MS analysis was performed at the Analytical Chemistry Unit, Faculty of Science, South Valley University (Table 2). The GC-MS analyses were carried out to detect the main components of RJ with an Agilent GC 6890 gas chromatograph coupled to an Agilent MSD 5973 mass detector in electron impact ionization mode. The gas chromatography column was a Zebron (ZB-1) methyl polysiloxane (30 m×0.25 mm i.d.×0.25 µm film thickness). The components of Chinese royal jelly were characterized by its Retention Time (RT) and determined by considering their areas as percentage of the total ion current (Table 2). The identification compounds were accomplished using computer searches on Wiley and NIST MS data library using MSD Chem Station software G170EA E.02.01.1177.

Broiler performance: The broilers were weighed every week and Feed Intake (FI) was measured weekly during the study. Daily body weight gain (g), daily feed consumption (g) per replication and feed conversion ratio of the chicks for the periods between days 0-21, 21-42 as well as for the overall

Table 2: Chemical composition of royal jelly bioactive components assessed by GC-MS

RT (min)	Contents	TIC (%)
Flavonoids		
36.337	Chrysin	0.847
33.502	Pinocembrin	1.842
35.114	Tectochrysin	0.382
32.176	Pinostrobin chalcone	0.735
Alcohol		
2.222	Furfuryl alcohol	0.279
Organic compounds		
8.871	Hydroxymethylfurfurole	0.656
Fatty acids		
16.924	3-hydroxydecanoic acid	1.494
19.463	10-hydroxydecanoic acid	19.815
32.398	Oleic acid amide	0.692
14.924	Octanoic acid, 8-hydroxy-(CAS)	3.226
Other		
2.896	2-penten-4-olide	4.110
5.751	4,5-diamino-2-hydroxypyrimidine	0.648
7.150	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1.409
3.891	Glutaconicanhydride	1.024
37.213	9-(4-Aminophenyl) acridine	0.489
42.011	Benzeneethanamine, N-[(4-nitrophenyl)methylene]	0.375
44.099	Ostreasterol	1.924
41.555	3-hydroxydiphenylamine	0.336
22.868	3-(4-nitrophenyl)propiolic acid trans-1,1-dichloro-2,3-diethylcyclopane	

RT: Retention time, TIC: The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation experiment were calculated. Dead birds were recorded every day to the end of the study (day 42) to calculate the mortality rate.

Behaviour measurements: Behavioural observations were performed throughout the 6 weeks experimental period using scanning techniques[17] in all groups was carried out at 2 min intervals during 2-20 min periods (10:00 to 10:20 and 14:00 to 14:20) for five days per week for six consecutive weeks. Before starting to observe a pen of birds, the observer sat quietly for 5 min to get the birds accustomed to his presence. Instantaneous scan sampling observations of chick's behavior were performed according to Lee and Craig[18]. For each scan the number of chicks engaged in eight mutually exclusive activities were recorded every 60 sec (feeding, drinking, resting, walking, standing, foraging, preening and feather pecking). A detailed description of those parameters is presented in Table 3. The percentage of chicks engaged in each behavior was calculated during all scan samples in each pen.

Blood samples collection and analysis: At the end of the experiment (day 42), bird per group were randomly selected from each treatment, slaughtered and then blood samples were collected. Blood samples were divided into two equal parts. The first part of blood was collected with heparin as anticoagulant to be used for counting of Heterophils (H), Lymphocytes (L) and H/L ratio according to Shen and Patterson[20]. The second part of each blood sample was centrifuged at 4000 rpm for 15 min to separate blood serum. The obtained serum was kept frozen at -20°C until analyzed. Serum samples were used to estimate serum biochemical by digital-VIS/ultraviolet spectrophotometer (723C visible spectrophotometer, Shanghai Phenix Optical Scientific Instrument Co., Ltd, China). Total serum proteins and albumin (g dL^{-1}) were assayed by a colorimetric method using a commercial kit as previously described[21]. Serum globulin was calculated by the difference between serum total protein and albumin since, the fibrinogen usually comprises a negligible fraction[22]. Also, the Albumin: Globulin ratio was calculated.

Statistical analysis: Results were analyzed statistically using SPSS (Statistical Package for the Social Sciences) version 16.0 for windows. The data were described using Means and Standard Deviations (SD) as descriptive statistics. Significant differences among treatment means were measured and compared by One-way analysis of variance (ANOVA) was performed followed by Least Significant Difference (LSD). Statistical difference with $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Effect of Chinese Royal Jelly (RJ) on performance of broiler chicken exposed to high stocking density: As shown in Table 4, daily body weight gain was similar among groups in the beginning of the study. This parameter was significantly lower in the HSD group at day 42 than in other groups ($p<0.05$). The RJ increased daily body weight gain in experimental groups to levels comparable to the control group. Daily feed consumption and feed conversion ratio were significantly deteriorated by HSD ($p<0.05$). Daily feed consumption and FCR improved in the HSD-RJ 250 and HSD-RJ 500 groups significantly ($p<0.05$). Mortality rates were significantly higher in the HSD group at day 42 than in other groups ($p<0.05$). The RJ supplementation also under HSD conditions, decreased the mortality rates of broilers resulting in levels similar to the control. The effects of poultry rearing under HSD conditions have been studied for years. Similar to the performance results in the present study, HSD was shown to cause decreased daily body weight gain, daily feed consumption and feed conversion ratio were significantly deteriorated by HSD, resulting in deteriorated poultry growth performance and welfare[15,23]. When the number of birds per unit of space increases, microclimate conditions around the birds deteriorate. The use of different management practices, equipment and several dietary alternatives has been recommended to alleviate such environmental stress[5,6]. The RJ has many flavonoids, organic compounds, fatty acids and other active ingredients as shown in Table 4. These chemical components can improve growth performance of broilers

Table 3: Behavioral ethogram of chicks according to Guo *et al.*[19]

Behavior	Description
Feeding	Head extended towards available feed resources while beak in or above the feeder appears to be manipulating or ingesting feed
Drinking	Beak and water contact or beak above drinker
Resting	Sitting, lying or sleeping with no other behavior
Walking	Progress in steps taken forward
Standing	The litter on the floor is not touching the bird's abdomen; all the while minimal other behavior is portrayed and bird is motionless
Foraging	Pecking and scratching the floor with feet
Preening	Beak related behavior that beak touches the plumage of the bird itself
Wing flapping	Wings are extended from the body and there is continuous flapping
Feather pecking	A broiler is active in pecking other bird's feathers

Table 4: Effect of different levels of Chinese Royal Jelly (RJ) on broilers performance at 0-6 weeks of age under different stocking densities

	Daily body weight gain (g)			Daily feed consumption (g)			Feed conversion ratio			Mortality rate		
	0-21	21-42	0-42	0-21	21-42	0-42	0-21	21-42	0-42	0-21	21-42	0-42
Treatments	(Days)											
Stocking density												
LSD (control)	43.54^{a}	653.09^{b}	1979.69^{c}	609.55^{ac}	1333.86^{b}	1935.16^{c}	2.16^{ad}	2.90^{a}	2.71^{a}	2.22^{b}	0.98^{bc}	1.67^{b}
HSD	43.65^{a}	654.77^{b}	1928.56^{d}	616.20^{a}	1334.28^{b}	1852.49^{d}	2.14^{a}	2.68^{be}	2.48^{c}	3.14^{a}	1.13^{b}	3.47^{a}
SEM	0.044	0.96	17.15	3.85	1.25	49.28	0.011	0.009	0.014	0.233	0.141	0.45
p-values	0.456	0.2396	0.001	0.003	0.6819	0.004	0.645	0.001	0.001	0.001	0.149	0.001
Stocking density×Royal jelly (RJ) level												
LSD-RJ 250	42.71^{ab}	650.74^{bc}	2093.35^{b}	608.03^{a}	1595.62^{a}	2203.65^{a}	2.13^{ad}	2.78^{b}	2.61^{ab}	1.87^{c}	1.12^{b}	1.25^{c}
LSD-RJ 500	43.50^{ab}	683.32^{a}	2246.36^{a}	639.82^{ad}	1376.38^{b}	2032.51^{b}	2.21^{ad}	2.82^{ab}	2.67^{ab}	1.82^{c}	0.98^{bc}	1.06^{c}
HSD-RJ 250	42.25^{ab}	657.77^{bd}	1902.78^{cd}	612.46^{ac}	1325.39^{b}	1839.25^{d}	1.90^{b}	2.33^{d}	2.18^{e}	2.01^{bc}	1.83^{ac}	3.21^{a}
HSD-RJ 500	41.98^{b}	659.59^{bd}	1955.77^{cd}	630.82^{ad}	1364.75^{b}	1899.41^{cd}	2.02^{ac}	2.45^{c}	2.27^{d}	1.94^{bc}	1.12^{bc}	1.57^{b}
SEM	2.43	5.71	51.38	13.86	42.26	61.31	0.13	0.064	0.072	0.179	0.092	0.129
p-values	0.527	0.001	0.001	0.001	0.001	0.001	0.216	0.001	0.001	0.285	0.001	0.002

Each mean represents 3 replicates for each treatment with each replicate containing 5 birds, $^{a-e}$Means in the same column and the same effect with different superscripts are significantly different (p<0.05)

Table 5: Influence of Chinese Royal Jelly (RJ) on total number of birds observed performing various behaviors (Mean%+SD) under different stocking densities

	Behavioral patterns							
Treatments	Feeding	Drinking	Resting	Walking	Standing	Foraging	Preening	Feather pecking
Stocking density								
LSD	14.86±1.29^{c}	5.86±1.09^{a}	39.06±3.48^{d}	11.47±1.32^{a}	13.73±1.86^{b}	4.85±0.77^{a}	8.75±2.31^{a}	1.94±0.048^{b}
HSD	6.04±1.48^{d}	2.89±1.67^{c}	55.20±2.30^{a}	6.30±1.42^{c}	16.38±2.98^{a}	4.08±0.56^{b}	6.90±0.47^{bc}	2.93±0.073^{a}
Stocking density×Royal jelly (RJ) level								
LSD-RJ 250	21.51±1.69^{b}	6.20±1.03^{ad}	45.09±3.11^{c}	8.65±0.68^{b}	9.64±1.12^{c}	3.05±0.37^{c}	6.59±0.57^{b}	0.27±0.015^{e}
LSD-RJ 500	23.73±1.62^{a}	6.87±1.02^{ad}	43.18±2.61^{c}	7.78±0.95^{b}	8.25±1.19^{c}	3.37±0.46^{cd}	7.54±0.63^{bc}	0.24±0.011^{e}
HSD-RJ 250	14.75±1.48^{c}	4.36±1.07^{be}	52.09±3.36^{b}	6.50±0.88^{c}	14.09±1.61^{bd}	2.47±0.61^{ce}	5.68±0.48^{bd}	1.01±0.036^{c}
HSD-RJ 500	15.36±1.56^{c}	4.87±1.26^{ae}	51.66±2.36^{b}	6.02±0.86^{c}	12.19±1.15^{be}	2.75±0.54^{ce}	5.77±0.52^{bd}	0.87±0.045^{d}
p-values								
Stocking density	0.001	0.001	0.001	0.001	0.007	0.004	0.005	0.001
Stocking density ×Royal Jelly (RJ)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Each mean represents 3 replicates for each treatment with each replicate containing 5 birds and $^{a-e}$Means in the same column and the same effect with different superscripts are significantly different (p<0.05)

under HSD stress resulting in increased daily body weight gain and daily feed consumption and better feed conversion ratio. The impact of these active ingredients and especially, flavonoids on bird performance under stress conditions was reported previously[6].

This result was in accordance with Pourali *et al.*[24] who found that garlic powder improved average daily feed intake in broiler chickens. In the same trend, Javed *et al.*[25] reported that feed intake was improved when broilers were supplemented with aqueous extract of medicinal plants containing garlic (10 mL L^{-1} of drinking water). Contrary, Aji *et al.*[26] mentioned that administration of 100 mg of garlic not affect feed intake of broiler chickens. Also, Ashayerizadeh *et al.*[27], Onibi *et al.*[28] and Mohebbifar and Torki[29] found no effect of garlic powder supplementation on feed intake of broiler chicks.

Effect of Chinese Royal Jelly (RJ) on behaviors of broiler chicken exposed to high stocking density: The effects of stocking density on broilers behaviour are presented in Table 5. The most commonly observed behaviour in all of the densities was resting (lying), the percentage of birds engaged is 39.06±3.48 to 55.20±2.30 in LSD compared to HSD, respectively. The percentage of birds engaged in feeding, drinking, walking, preening and foraging behavior in HSD were significantly lower than the LSD group (p<0.05). Standing and feather pecking behaviour tended to increase in HSD than LSD group of all experimental period. Other studies have shown that as stocking densities increase, chickens spend less time resting, suggesting that an increase in animal density results in decreased opportunities for undisturbed rest[3,4,30]. Stocking density affected standing behaviour in Table 5, it seems to affect the increase in the level of tension[31-33].

The effects of dietary supplementation of Chinese Royal Jelly (RJ) on broilers behaviors are presented in Table 5. A significantly higher proportion of chicks in RJ supplementation, LSD-RJ 250 and LSD-RJ 500 were engaged in feeding behavior 21.51±1.69 and 23.73±1.62 compared to control group14.86±1.29 (Mean±SD, respectively p = 0.001) with HSD group being also increased proportion of chicks engaged in feeding behavior in RJ supplementation but not different from control group (14.75±1.48 and 15.36±1.56) in LSD-RJ 250 and LSD-RJ 500, respectively which in turn was reflected in final body weight and other productive performance of chicks. Results from behavioral observations in the present study indicated that dietary supplementation of Chinese Royal Jelly (RJ) significantly affects the proportion of birds engaged in resting, walking, standing, foraging and preening behaviors (Table 5). Birds in unsupplemented diet control group were showed significantly less resting behavior 39.06±3.48 compared to birds in LSD-RJ 250 and LSD-RJ 500 groups; 45.09±3.11and 43.18±2.61, respectively (Mean±SD p = 0.001). With HSD group being also increased proportion of chicks engaged in resting behavior in RJ supplementation that differ from control group (52.09±3.36 and 51.66±2.36) in HSD-RJ 250 and HSD-RJ 500, respectively. Results summarized in Table 5 showed non-significant differences of dietary treatment on the percentage of birds engaged in drinking. Increased standing behaviors in control and HSD birds may be related to low feeding behavior that demonstrated in these groups. Hocking *et al.*[34] reported that pacing was negatively related to rate of consumption. Moreover, Hocking[35] observed that the proportion of time spent standing and walking was associated with a decrease in the proportion of time involved in eating, scratching and pecking activities.

As shown in Table 5, Percentage of birds engaged in walking behavior, significantly decreased in the HSD group ($p<0.001$) resulting in locomotion problems and difficulty accessing feeders and drinkers. The RJ supplementation decrease the number of the birds engaged in walking behavior in both doses also under HSD conditions, the number of the birds decreased, resulting in levels similar to the control. The Percentage of birds engaged in standing were significantly higher in the HSD group, HSD-RJ 250 and HSD-RJ 500 groups than in the LSD, HSD-RJ 250 and HSD-RJ 500 groups ($p<0.05$). The birds have to spend more time standing which results in social anarchy for resting birds[36]. All of these problems cause physical and physiological stress to the birds[37]. The total percentage of birds engaged in foraging and preening were higher in the control than HSD, LSD-RJ 250, LSD-RJ 500, HSD-RJ 250 and LSD-RJ 500 groups ($p<0.05$). The Percentage of birds engaged in feather pecking behavior were significantly higher in the HSD group than in the LSD whereas, RJ supplementation under LSD (LSD-RJ 250, LSD-RJ 500) and HSD (HSD-RJ 250, HSD-RJ 500) conditions significantly decreased the percentage of birds engaged in feather pecking behavior ($p<0.001$).

Effect of Chinese Royal Jelly (RJ) on serum biochemical parameters of broiler chicken exposed to high stocking density: Results presented in Table 6 indicated that serum total protein, albumin and globulin concentration were similar among LSD and HSD groups. The RJ treatment significantly increased serum total protein, albumin and globulin concentration and this effect was dose-dependent ($p<0.05$). Adding RJ to the diet of stressed broilers was reflected with significant higher contents of its serum total protein, albumin and total globulin in the HSD-RJ 500 groups ($p<0.05$). The

Table 6: Effect of different levels of Chinese Royal Jelly (RJ) on hematological properties portrayed by the chicks at 6 weeks of age under different stocking densities

	Blood parameters					
Treatments	Total protein (g/100 mL)	Albumin (g/100 mL)	Globulin (g/100 mL)	H (10^3/100 mL)	L (10^3/100 mL)	H/L ratio
Stocking density						
LSD (control)	4.34 ± 0.59^{c}	2.44 ± 0.04^{c}	1.84 ± 0.13^{bcf}	20.60 ± 3.79^{b}	34.60 ± 3.12^{bc}	0.57 ± 0.01^{b}
HSD	4.09 ± 0.35^{cd}	2.48 ± 0.06^{c}	1.75 ± 0.11^{c}	25.86 ± 3.87^{a}	32.65 ± 2.33^{b}	0.77 ± 0.06^{a}
Stocking density×royal Jelly (RJ) level						
LSD-RJ 250	4.54 ± 0.21^{ce}	2.54 ± 0.01^{b}	1.94 ± 0.10^{bc}	17.53 ± 2.23^{c}	38.29 ± 2.24^{a}	0.50 ± 0.05^{c}
LSD-RJ 500	5.24 ± 0.32^{a}	2.84 ± 0.05^{a}	2.34 ± 0.01^{a}	18.19 ± 2.04^{c}	38.57 ± 2.51^{a}	0.51 ± 0.02^{c}
HSD-RJ 250	4.56 ± 0.23^{ce}	2.45 ± 0.03^{c}	2.01 ± 0.12^{bce}	20.45 ± 2.01^{bc}	36.18 ± 2.82^{ac}	0.46 ± 0.04^{c}
HSD-RJ 500	4.90 ± 0.06^{b}	2.47 ± 0.02^{c}	2.26 ± 0.19^{a}	21.13 ± 2.05^{bc}	37.83 ± 2.30^{a}	0.49 ± 0.08^{c}
p-values						
Stocking density	0.169	0.782	0.0502	0.003	0.0626	0.001
Stocking Density×Royal Jelly (RJ)	0.001	0.001	0.001	0.001	0.0486	0.002

Each mean represents 3 replicates for each treatment with each replicate containing 5 birds, H: Heterophils, L: Lymphocytes and [a-e]Means in the same column with different superscripts are significantly different ($p<0.05$)

results are comparable to Kurkure *et al.*[38] reported an increase in plasma albumin concentration in White Leghorn cockerels, orally treated with RJ at 10 mL $bird^{-1}$ day^{-1}. The results of Elnagar *et al.*[39] who reported that in stressed male rabbits, RJ led to a significantly increase in serum total protein, albumin and globulin. In contrast, RJ did not influence serum total protein, albumin and globulin in growing rabbits[39]. Meanwhile, the improvement of serum total protein and its fractions in the group fed RJ may be related to its direct effect as a growth promoter on the haemopoietic tissue and the stimulating effect on the liver exhibiting an anabolic action favoring protein synthesis and also its preserving effect on the body protein from degeneration[39].

The present study showed that HSD groups had significantly higher heterophil and H/L ratio compared to control group ($p<0.05$), while the lymphocytes was not significantly affected by stocking density. Meanwhile, groups fed RJ (LSD-RJ 250, LSD-RJ 500) had significantly lower heterophil and H/L ratio compared to control group ($p = 0.001$). The HSD groups shown an increase in lymphocytes compared to (HSD-RJ 250, HSD-RJ 500) ($p<0.05$). The increased percent of lymphocytes in broilers fed diets with RJ may be related to its effect as antibacterial, antiviral and antifungal on their immunity system.

The significantly lower heterophil in RJ supplemented birds, these results indicated that control and HSD groups were more stressed and supplementation of diet with RJ reduced stress in broiler chicks. Maxwell[40] mentioned that stressed birds shown an increase in H/L ratio and this ratio may be a more reliable indicator of mild to moderate stress than plasma cortisone concentration. Stress in HSD birds was reflected on its productivity. These results were in accordance with Saxena and Madan[41] who reported that stress evokes harmful responses that interferes with the general health, productivity and resulted in immunosuppression. Results of this study were in agreement with Al-Kassie[42] and Najafi and Torki[43] they reported that groups fed oil extract derived from thyme had significantly lower H/L ratio. Also, Ali[44] found supplementation of the broiler chickens diet with *Thymus vulgaris* leaves powder from 0-8 weeks of age resulted in significant ($p<0.01$) decrease in heterophil to lymphocyte ratio.

CONCLUSION

In conclusion, the RJ is regarded as a valuable food supplement because of its functional, biological and pharmaceutical properties. This study suggest the potential protective activity of this bee product on performance, behaviour and blood parameters of broilers under HSD stress.

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