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Effect of supplementation of synbiotic plus betaine and selenium on behavior and performance of broilers stocked at high stocking density pens

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Abstract This experiment aimed to examine the effect of synbiotic plus betaine and selenium supplementation on the behavior, blood profile and performance of broiler chickens reared at stocking density pens. For 21 days, a total of 330 broilers with body weight of 929.67 ± 21.15 g were randomly assigned to 35 experimental units (pens) with a normal stocking density (NSD) of 9 birds/m^2 as a positive control and high stocking density (HSD) of 15 birds/m^2. A total of 7 treatments were applied, consisting of 1 NSD treatment (CONTR-) and 6 HSD treatments, i.e., CONTR+ (positive control); SYN (1% synbiotic), BET (betaine 2 g/kg feed), SYNBET (synbiotic 1% + betaine 2 g/kg feed); SE (selenium 0.4 mg/kg feed); SYNSE (1% synbiotic + selenium 0.4 mg/kg). Parameters including behavior, blood profile and performance were measured at week 4 (d 28) and week 5 (d 35). The results showed that the supplementation of synbiotic, betaine, selenium, or their combination improved ($P< 0.05$) the behavior, blood profile (eritrocytes, hemoglobin) and performance of broiler chickens at high stocking densities. The conclusion of the study was synbiotic plus betaine (SYNBE) supplementation can improve behavior and produce the best performance in broilers reared at high stocking densities.

Keywords: behaviour, blood, growth, *Lactobacillus casei*

1. Introduction

Stocking density is an important factor in broiler production because it affects the health, welfare, behavior, and performance of poultry (Sugiharto 2022). According to Abudabos et al (2013), the European Commission of Ethics in 2007 applied a minimum standard of broiler welfare with a maximum stocking density of 30 kg/m^2 (0.073 m^2/bird). In general, high stocking density (HSD) causes behavioral changes and physiological stress (Thomas et al 2011; Hall 2001). Previous studies have also reported that high stocking densities can affect the performance and behavior of chickens (Eugen et al 2019; Li et al 2019), decreased feed intake, increased drinking frequency (Ibrahim et al 2018), increased rest, decreased movement activity, decreased frequency of pecking and preening patterns (Simitzis et al 2012; Hall 2001). When livestock's homeostasis is threatened, they develop stress as a biological reaction (Virden and Kidd 2009). Indeed, chickens walk and stand less, consume less feed and drink more water, spread their wings and cover their bodies on the surface of the litter, and pant when stressed (Wasti et al 2020).

Stress causes damage to the intestinal mucosal epithelial microstructure, oxidative damage, worsens the immune system, and increases susceptibility to intestinal barrier integrity (Virden and Kidd 2009). This causes the invasion of pathogenic microbes and causes systematic inflammation, decreased immune response, interferes with the activity of nutritional digestive enzymes, and reduces production performance in poultry (Patra and Kar 2021). It has been well documented that rearing at high stocking densities can cause physiological stress and impaired immune competence in broilers (Simitzis et al 2012). High stocking density causes oxidative stress as seen from decreased levels of glutathione (GSH) and increased levels of malondialdehyde (MDA) in liver tissue, lowering serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Li et al 2019).The combination of probiotics and prebiotics, referred to as synbiotics, has usually been used to improve gut health, immune system, and avoid physiological stress in broilers (Sohail et al 2013; Ashraf et al 2012; Cengiz et al 2015). Supplementing with betaine and selenium is another attempt to minimize the negative effects of stress. The osmolyte activity of betaine is helpful to the intestinal epithelium because it helps maintain villous integrity, which enhances digestion and nutritional absorption (Kettunen et al 2001; Eklund et al 2005; Ratriyanto and Pratowo 2019). Selenium is a cofactor in the glutathione peroxidase (GSH-Px) enzyme system, which regulates hydroperoxides both outside and inside the cell. Selenium has the ability to protect tissues from oxidative damage and can boost the immune system (Wang et al 2021; Ahmadi et al 2018; Rao et al 2016; Chen et al 2014). Based on these previous findings, the study was conducted to assess the effect of synbiotic plus betaine and selenium supplementation on broiler behavior and performance in high stocking density.

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2. Materials and methods

2.1. Synbiotic Preparation

Synbiotic production was carried out according to Mangisah et al (2021). In short, *Lactobacillus casei* bacteria (concentration of 10^9 cfu/ml) were then inoculated into 500 ml of skim milk solution (10 g skim milk dissolved in 100 g distilled water) and incubated (24 hours at the temperature 37 ºC). The incubation results were added with 1.5% garlic powder and then incubated again at the same temperature for 48 hours. Synbiotic was stored in the refrigerator.

2.2. Animals and Feeds Experiment

All procedures used in this experiment were approved by the Animal Research Ethics Committee, Faculty of Animal Husbandry and Agriculture, Diponegoro University No. 57-01/A-4/KEP-FPP. In this experiment, 330 one-day male broilers CP 707 were used with an initial body weight of 49.61 grams from a commercial hatchery. Chickens were placed randomly in cages with length, width, and height of 80, 80, and 100 cm, respectively. Broilers were reared in open cage systems under natural tropical conditions. Feed and water were given *ad libitum*, with continuous lighting throughout the experiment. The starter diet was fed from 1 to 21 days and the finisher diet was fed at 22 to 35 days (Table 1) Seven treatments were repeated 5 times, starting on day 21 onward. The research treatments included CONTR- (normal stocking density with 9 chickens/m^2); CONTR+ (positive control HSD with 15 chickens/m^2 without supplementation); SYN (15 bird/m^2 rearing HSD with 1% synbiotic supplementation); BET (15 bird/m^2 rearing HSD with betaine 2 g/kg feed supplementation), SYNBET (15 bird/m^2 rearing HSD with supplementation of 1% synbiotic + betaine 2 g/kg feed); SE (15 bird/m^2 rearing HSD with supplementation of selenium 0.4 mg/kg feed); SYNSE (15 bird/m^2 rearing HSD with supplementation of 1% synbiotic + selenium 0.4 mg/kg). On days 4 and 18, broiler chickens were intraocularly vaccinated against Newcastle disease and on day 12, Gumboro vaccine was delivered by drinking water. The synbiotic used in this study was a mixture of *Lactobacillus casei* and garlic powder. The process of making synbiotics refered to the procedure described by Mangisah et al (2021). Betaine used was 98% betaine HCL for PT Groviter Jakarta Indonesia commercial feed products.

Table 1 Research feed and nutrient content.

Ingredient (%, except that otherwise mentioned)	Starter (1 -21)	Finisher (22-35)
Yellow corn	56.00	63.40
Soybean meal	36.90	30.00
Palm oil	2.30	2.30
Bentonite	1.00	0.50
Limestone	1.33	1.33
Mono Calcium Phospat	1.45	1.45
Premix*	0.57	0.57
NaCl	0.35	0.35
Total	100	100
Analyzed nutritional compositions		
ME (kcal/kg)	2918	3003
Dry matter	86.35	87.15
Crude protein	21.8	19.16
Ether extract	2.61	2.82
Crude fiber	3.32	3.21
Calcium	0.85	0.87
Phosphor	0.42	0.49

2.3. Measurements of Parameter

Feed consumption, body weight gain, and FCR were measured weekly, on week 4th (d 28) and 5th (d 35). On days 28 and 35, data on broiler behavior was obtained using the focal sampling method by recording eating, drinking, and breathing activity in 35 experimental units for 15 minutes each at 07:00, 12:00, and 17:00. Calculating the frequency of pecking, drinking, and breathing for 15 minutes, captured every 30 seconds, was used to make observations on the film. This ensured that the computation findings were more accurate (Prayitno and Sugiharto 2015). The average per minute for each replication of each treatment was obtained after the observations were totaled up. On days 28 and 35, the average frequency of eating, drinking, and breathing behavior of broiler chicks was calculated by adding the findings from the morning, afternoon, and evening observations and dividing by three.

The protocol for the tonic immobility test had previously been published (Mohammed et al 2021). On days 28 and 35 of 70 broiler chickens, tonic immobility data were collected, with two birds sampled in each replication. The average of each replication in the treatment group was computed using the results obtained from calculating tonic immobility for two samples of broiler chickens. To induce a state of tonic immobility, birds were placed upside down in a cradle and held for 5 seconds with light pressure. The duration of immobility was measured once the pressure was released.

A sample of 35 broilers was taken from pen cage. Blood was taken on day 35 from the wing vein. The blood samples were analysed using a hematology analyzer referring to the instructions from PT. Prima Alkesindo Nusantara, Indonesia. Blood data includes the number of leucocytes, total protein plasma, monocytes, eritrocytes, hemoglobin (Hb), heterophils, lymphocytes and hematocrit.

2.4. Statistical Analysis

The research used a completely randomized design. The data obtained were analyzed by ANOVA and Duncan's test with SPSS version 22.

3. Results and discussion

The results of the study of supplementation synbiotics, betaine, and selenium significantly (P<0.05) on behavior, blood profile and performance were presented in Tables 2, 3 and 4. Raising chickens at high density (HSD) decreased feeding behavior and increased drinking behavior. This is shown by the data on Table 2, CONT+ both at the age of 28 and 35 days, compared to CONT-. The decrease in feeding frequency in the HSD group at the age of 28 and 35 days was due to the limited space to access feed, resulting in competition between birds for feed (Sugiharto 2022). In addition, it seems that air quality and an increase in environmental temperature (the average temperature 35ºC during the day) cause stress which has an impact on decreasing the frequency of eating and increasing the frequency of drinking. High stocking densities can affect productivity and behavior of hens (Eugen et al 2019; Li et al 2019), decreased feed consumption, increase drinking frequency (Ibrahim et al 2018).

Table 2 Behavior of eating, drinking, and breathing frequency of broiler chickens.

Items	Variable (times/minutes)	Treatments							SEM	*p*
		CONTR -	CONTR+	SYN	BET	SYNBET	SE	SYNSE		
Day-28	Eating Frequency	9.23^{a}	7.88^{b}	8.24^{b}	8.18^{b}	8.09^{b}	9.01^{a}	8.16^{b}	0.07	<0.001
	Drinking Frequency	3.58^{c}	3.79^{bc}	4.1^{a}	4.22^{a}	4.03^{ab}	4.29^{a}	4.08^{a}	0.32	<0.001
	Breathing Frequency	80.93^{d}	83.86^{ab}	84.95^{ab}	82.08^{c}	85.34^{a}	83.14^{bc}	82.04^{c}	0.23	<0.001
	Tonic immobility	1.41^{b}	1.60^{a}	1.49^{ab}	1.60^{a}	1.50^{ab}	1.53^{ab}	1.49^{ab}	0.2	0.136
	Eating Frequency	9.46^{a}	8.05^{b}	9.37^{a}	8.23^{b}	8.09^{b}	8.42^{b}	8.49^{b}	0.07	<0.001
Day-35	Drinking Frequency	3.4^{d}	4.55^{a}	3.55^{d}	3.89^{c}	3.99^{c}	4.34^{ab}	4.26^{b}	0.31	<0.001
	Breathing Frequency	83.69^{c}	88.98^{a}	85.05^{bc}	83.88^{c}	85.89^{bc}	87.35^{ab}	87.27^{ab}	0.32	0.001
	Tonic immobility	1.48^{f}	1.87^{a}	1.81^{ab}	1.78^{bc}	1.64^{de}	1.72^{cd}	1.58^{e}	0.01	<0.001

Notes: Superscripts with different letters in the same line are significantly different (P<0.05).

Administration of selenium (Se) was able to improve feeding behavior in 28-day-old chickens reared on HSD. The addition of selenium in feed can reduce the detrimental effects of heat stress (Wang et al 2021; Ahmadi et al 2018). Selenium is an important component of enzymes such as glutathione peroxidase (GSH-Px), which functions in antioxidant protection systems (Wang et al 2021). The protective action of Se in chickens with high stocking density has been shown to increase antioxidant capacity, modulate immune function, prevent inflammation and thus reduce the negative effects caused by HSD (Liao et al 2012; Safdari-Rostamabad et al 2017; Sun et al 2021). This affects the eating behavior of chickens as indicated by an increase in the frequency of feeding in the high density group with selenium supplementation (SE).

Meanwhile, the addition of synbiotics (SYN) was able to improve the feeding behavior of high-density reared chickens on the 35th day. Synbiotics can improve the balance of intestinal microbes and the immune system to reduce the effects of stress (Ashraf et al 2013; Cengiz et al 2015). It has been reported that probiotic and synbiotic supplementation can improve the stress condition of broilers reared in HSD. Giving synbiotics to HSD chickens was able to improve the microbial ecosystem (Kridtayopas et al 2019) and the morphology of the intestine (Altaf et al 2019). Morphological improvement is seen from the increase in the number of goblet cells so that the production of intestinal mucus increases and consequently improves the integrity and barrier of the intestine. Healthy gut conditions, in turn, improve the performance of organs and digestive enzymes, the utilization of nutrients by HSD broilers also increases. This is thought to be the cause of the increase in the frequency of eating in this study (SYN).

HSD also significantly (P<0.05) increased the frequency of breath, both in chickens aged 28 and 35 days. These results are by the findings of Zabir et al 2021, that with increasing rearing age (3 weeks), high-density chickens (SD2.0 and SD2.5) showed an increase in the frequency of breathing and panting during the day (11.00 pm until 4 pm). The increase in the frequency of breathing is carried out by the chickens to maintain body temperature because the livestock suffers from stress due to the increase in the temperature of the cage caused by the high stocking density (the average temperature is 35ºC during the day). Three-week-old broilers should be kept in an environment with temperatures ranging from 20-25oC and relative humidity around 50-70% and 26-27°C for adult broilers (Tamzil 2014). The increase in respiratory frequency was due to heat stress due to HSD. When livestock suffers from stress, the system directly activated neurogenic (Virden and Kidd 2009), which in the alarm phase is marked by an increase in blood pressure, muscle, nerve sensitivity, blood sugar, and respiration. If these efforts fail to cope with stress, then the body will activate the hypothalamic-pituitary-adrenal cortical system. When this system is activated, the hypothalamus produces corticotrophin-releasing factor, which in turn stimulates the pituitary for the release of adrenocorticotropic hormone (ACTH). Previous research reported that an increase in ambient temperature markedly increases the temperature body (Tamzil 2014). Maintenance in cage temperature when the ambient temperature reaches 40oC and is left for 1.5 hours, the rectal temperature increases to 44.99°C accompanied by an increase in the frequency of panting, water consumption drinking and decreased feed consumption (Tamzil 2014). Administration of sinbiotik (SYN), betaine (BET), synbiotik+betaine (SYNBET), selenium (SE), and sinbiotik+selenium (SYNSE) was able to decrease the respiratory rate in chickens reared with HSD on the 28th and 35th day. All of the additives given improved respiratory rate, despite not matching the chickens in the CONTR- (normal density). Observation of broiler breathing frequency on the 35th day was the highest indicated by CONTR+ (HSD without supplementation) when compared to other treatment groups. Changes in behavior patterns to dissipate heat through evaporation from the respiratory tract or panting are to overcome heat stress caused by high-density rearing (Prayitno and Sugiharto, 2015). In high-density chickens treated with additives such as sinbiotik (SYN), betaine (BET), synbiotik+betaine (SYNBET), selenium (SE), and sinbiotik+selenium (SYNSE) they had a lower possibility of oxidative stress so that the respiratory rate was lower than the CONT- group. This was also seen from the performance. For the record, chickens at 35 days of age had a higher potential for heat stress than those on 28 days, so the results of observing the respiratory frequency on the two days showed different tendencies. This was also seen from the tonic immobility data. High stocking density increases the duration of tonic immobility (Sugiharto 2022) and decreases locomotor activity (Simitzis et al 2012). Tonic immobility in chickens aged 28 days and 35 which were kept in HSD conditions showed better values with the provision of various additives, both synbiotic (SYN), betaine (BET), a mixture of synbiotic+betaine (SYNBET), selenium (SE), and synbiotic+Selenium (SYNSE), compared with HSD (CONTR+). Administration of SYNBET and SYNSE showed the most effective effect in improving tonic immobility.

Results in Table 3 showed that the erythrocyte, hemoglobin and blood hematocrit levels of chickens reared at high cage density (HSD) were significantly lower (P<0.05) than normal density (NSD). Meanwhile, administration of SYN, SYNBET, and SYNSE produced the same erythrocyte values. This is due to the contribution of synbiotics which can improve the acidity of the digestive tract, so that the balance of intestinal bacteria becomes better. The fermentation process by *Lactobacillus* in synbiotics, causes an increase in the activity of digestive enzymes, helps better absorption of protein, iron and production of vitamin B complex, which has a positive effect on the blood formation process. According to Beski and Al-Sardary (2015), that iron availability is closely related to the formation of red blood cells and hemoglobin.

Table 3 Blood profile of broiler chickens on day 35.

Variable	Treatments							SEM	*p*
	CONTR -	CONTR+	SYN	BET	SYNBET	SE	SYNSE		
Erythrocytes (10^6/µL)	$2,26^{ab}$	$1,97^{c}$	$2,37^{a}$	$2,16^{b}$	$2,32^{a}$	$2,11^{b}$	$2,37^{a}$	0,05	0,05
Leukocytes (10^3/µL)	10,24	10,15	10,19	10,87	10,67	10,17	10,20	0,30	0,92
Total plasma protein (g/dL)	30,35	28,71	29,81	31,09	31,60	27,01	28,81	0,55	0,51
Heterophile (10^3/µL)	29,20	35,60	27,40	30,60	30,77	28,20	29,40	1,57	0,57
Lymphocytes (10^3/µL)	72,00	68,40	68,20	69,20	67,00	68,70	66,20	1,00	0,60
Monocytes (10^3/µL)	2,40	2,90	2,60	2,70	2,20	2,75	2,60	0,54	0,74
Hemoglobin (g/dL)	$8,06^{a}$	$7,08^{b}$	$7,48^{b}$	$8,04^{a}$	$8,14^{a}$	$7,38^{b}$	$7,48^{b}$	0,08	0,01
Hematocrit (%)	$27,80^{ab}$	$22,00^{c}$	$26,40^{b}$	$25,60^{b}$	$29,60^{a}$	$25,40^{b}$	$26,47^{b}$	0,48	0,01

Notes: Superscripts with different letters in the same line are significantly different (P<0.05).

Administration of betaine alone or selenium alone produced better erythrocyte values compared to the positive control, but the values were SYN, SYNBET, and SYNSE. It can be explained here that betaine and selenium can be used to overcome stress caused by high cage density, but do not provide improvements in the composition of intestinal microbes, which affect the availability of nutrients as raw materials for the synthesis of blood erythrocytes.

Abudabos et al. (2013) that rearing broiler chickens at a density of 28 to 40 kg/m2 resulted in a significant decrease in hematocrit. The decrease in the number of erythrocytes seen in HSD affects the decrease in Hb and hematocrit. Erythrocytes function to carry oxygen, so the amount of oxygen in the blood decreases and the decrease in erythrocytes also causes hemodilution (blood thinning), which causes the hematocrit to decrease in HSD. Apart from the negative effects on the above parameters, rearing at high cage density (HSD) did not have an effect on the parameters of leukocytes, total plasma protein, heterophils, lymphocytes, total cholesterol and high density lipoprotein (HDL). Several previous researchers also did not find the same results, that there was no effect whatsoever from high cage density on the physiological of broilers. he levels of cholesterol, corticosterone, glucose, heterophils and blood lymphocytes of broiler chickens reared in high cage density were as good as controls. (Houshmand et al 2012).

When broilers are kept at high stocking densities, there is a reduction in the space for movement because it is getting narrower, and there is an increase in the temperature in the cage (up to 35°C). This condition causes stress so that chickens reduce the formation of body heat by reducing activities in cage and basal metabolic rate by reducing the frequency of eating, increasing the frequency of drinking, and the frequency of breathing. Chickens spend more time lying down and sleeping, so feed consumption is reduced. The data of this study showed that the feed intake of chickens with high stocking densities was significantly lower than normal stocking densities, both at 28 and 35 days of age. A previous study showed that two weeks of heat stress in broilers caused daily feed intake to decrease by 2.67% (Li et al 2000). Changes in behavior and low feed intake in high stocking density chickens have an impact on performance. The performance of CONT+ (HSD without supplementation) chickens was worse than CONTR- (NSD) and HSD with additive supplementation. The results of this study are in line with Asaniyan and Akinduro (2021) that broilers reared at HSD 15 bird/m^2 had the lowest growth compared to those reared at NSD 10 bird/m^2 and 5 bird/m^2.

The results of the study of supplementation synbiotics, betaine, and selenium significantly (P<0.05) on behavior, blood profile and performance were presented in Tables 4. Initial body weight and BWG in 28-day-old chickens that were given synbiotic+betain and Synbiotic+Selenium (SYNBET, SYNSE) were significantly higher (P<0.05) compared to other treatments in the HSD group. However, in chickens given only synbiotics, initial body weight and BWG were still low and different from SYNBET AND SYNSE, even the same as CONTR+ (without supplementation). This is presumably because the synbiotic effect is more focused on improving the balance of microbes, intestinal villi and digestive tract health alone, but for the improvement of antioxidant capacity as a free radical scavenger, the synbiotics combined with betaine and selenium (SYNBET and SYNSE) have synergies. synbiotic work with Betaine and Selenium performance as an antidote to stress because it improves antioxidant activity and the body's immune system (Kettunen et al 2001; Eklund et al 2005; Ratriyanto and Pratowo, 2019), although data on antioxidant activity and immunity were not reported in this study.

The results of the performance of HSD chickens aged 35 days showed a slightly different phenomenon from the age of 28 days. Due to increasing age, causing the chickens to get bigger, the space to move is more limited and the heat conditions in the cage are getting higher and the litter conditions are getting wetter. The 35th day observation showed that the addition of synbiotic combined with betaine (SYNBET) was able to produce the best final body weight, BWG, and FCR. The administration of synbiotics (a combination of prebiotics and probiotics) in chickens experiencing heat stress due to high cage density can reduce some of the adverse effects on the gut microstructure of broilers increase the surface area and depth of the jejunum crypt (Kridtayopas et al. 2019; Altaf et al 2019)), improve beneficial bacterial populations in the gut (Sohail et al 2013), thereby increasing nutrient digestibility (Mangisah et al 2021) and increasing body weight (Sohail et al 2013). Synbiotics work synergistically with betaine, which is able to increase nutrient absorption by widening the surface of the chicken intestine and improve blood profile (Erythrocytes, hemoglobin and hematocrit). Betaine has 3 methyl groups that can be donated in the transmethylation process to be accepted by the acceptor, so the presence of betaine reduces the use of methionine as a methyl donor, so that methionine in feed is utilized for the synthesis of body tissues rather than as a methyl group donor (Ratriyanto et al., 2009). In addition, betaine as an osmolyte (cell protection from osmotic pressure), the zwitterionic characteristic of betaine helps maintain cell water homeostasis (Klasing et al., 2002) without affecting cell metabolism. Several studies have shown that betaine supplementation is beneficial for intestinal epithelium, osmolyte function maintained villi integrity and consequently increasing digestibility and absorption of nutrients (Kettunen et al., 2001; Eklund et al., 2005). The addition of betaine improves the height of the duodenal and jejunal villi due to heat stress maintenance, thereby increasing nutrient intake and BWG in broiler chickens (Liu et al 2019). The group of chickens treated with betaine supplementation showed a significant increase in glutathione peroxidase activity parameters (Alirezaei et al 2012). Increased activity of antioxidant enzymes with betaine has been shown to reduce the adverse effects of long-term heat stress on growth performance, digestive function, and carcass properties in broiler chickens (Liu et al 2019). Reduction of the adverse effects of stress on HSD conditions due to the administration of SYNBET, in FI, Initial body weight, BWG and FCR.

Table 4 Broiler chicken performance.

Items	Variable	Treatments							SEM	*p*
		CONTR -	CONTR+	SYN	BET	SYNBET	SE	SYNSE		
Day-28	FI (g/bird/week)	960.92^{a}	780.02^{cd}	726.46^{d}	899.86^{ab}	810.86^{bcd}	767.08^{cd}	848.2^{bc}	12.49	<0.001
	Initial body weight (g)	1592.64^{a}	1412.72^{c}	1422.02^{c}	1494.28^{b}	1498.9^{b}	1409.78^{c}	1494.24^{b}	7.24	<0.001
	BWG (g/bird/week)	636.63^{a}	456.71^{c}	466.01^{c}	538.27^{b}	542.89^{b}	452.77^{c}	538.23^{b}	7.24	0.522
	FCR	1.52^{b}	1.74^{a}	1.58^{b}	1.67^{a}	1.49^{b}	1.7^{a}	1.59^{b}	0.04	<0.001
	FI (g/bird/week)	1206.18^{a}	1103.2^{bc}	1160.34^{ab}	1036.5^{c}	1122.48^{ab}	1073.06^{bc}	1087.62^{bc}	12.45	0.023
Day-35	Initial body weight (g)	2378.8^{a}	2006.22^{d}	2204.2^{bc}	2142.4^{bc}	2272.8^{ab}	2094^{cd}	2236^{b}	15.94	<0.001
	BWG (g/bird/week)	786.16^{a}	633.4^{b}	782.18^{a}	648.12^{ab}	773.9^{a}	684.22^{ab}	741.76^{ab}	16.04	0.056
	FCR	1.55^{ab}	1.88^{a}	1.49^{b}	1.69^{a}	1.45^{b}	1.61^{ab}	1.48^{b}	0.05	0.177

Notes: Superscripts with different letters in the same line are significantly different (P<0.05).

4. Conclusions

Synbiotic plus betaine (SYNBET) supplementation can improve behavior and produce the best performance in broilers reared at high stocking densities.

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Ethical considerations

All procedures used in this experiment were approved by the Animal Research Ethics Committee, Faculty of Animal Husbandry and Agriculture, Diponegoro University No. 57-01/A-4/KEP-FPP

Conflict of interest

There is no any conflict of interest in the manuscript.

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