Research Article

The Effect of High Stocking Density and Dietary Protein Levels on Blood Profiles, Intestinal Bacteria, Some Immunological Parameters, Status Antioxidant, and Performance in Native Chickens

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Abstract | This study aimed to investigate the effects of high stocking density and crude protein levels on blood parameters, bacterial populations, immune organs, antioxidant status, and growth performance in Sentul Selection (SenSi) 1 Agrinak chickens. Treatments consisted of three stocking densities (10, 14, and 18 birds/m^2) and three levels of crude protein (14, 16, and 18%) factorially (3×3) which were arranged in nine treatments and four replications. Treatment and data collection were carried out at the age of 29-70 days. Data were analyzed using ANOVA with a factorial Group Random Design. The results showed that stocking density and crude protein levels had no impact on the blood profile, number of lactic acid bacteria (LAB) and coliforms in the cecum, weight of the bursa fabricius and spleen, levels of malondialdehyde (MDA), number of Newcastle Disease Virus (NDV) antibodies, increase in body weight, and feed efficiency. High stocking density has a significant impact on increasing the number of LAB and coliforms in the ileum, reducing the weight of the thymus, and reducing the amount of feed consumed. The lower the crude protein level, the lower the NDV antibody titres, but it did not affect the blood profile, LAB and coliform numbers, lymphoids, MDA levels, or growth performance. It was concluded that high stocking densities and reduced crude protein levels have the potential to harm health but do not affect the growth performance of Sensi 1 Agrinak chickens.

Keywords | Sentul Selection 1 Agrinak Chicken, High Stocking Density, Dietary Crude Protein Level, Gut Health, Immunity, Performance

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INTRODUCTION

Global poultry meat demand in 2032 is projected to increase to 91 million tons of retail weight equivalent (rwe), prompting poultry producers to increase meat production. However, the challenge for meat producers to increase production is the high cost of feed and labor (OECD, 2023). Some of the strategies currently used by livestock companies include maximizing stocking density and efficient feed utilization. On farms, stocking density (SD) is a critical component that affects financial returns for producers and welfare for birds, while feed utilization efficiency in poultry production can contribute to reducing negative impacts on biodiversity and positively affect the

economic sustainability of the poultry chain (Zampiga et al., 2021; Shynkaruk et al., 2023).

High stocking densities (HSD) have a positive effect on economic returns but can lead to increased temperature in the cage, scrambling for food, increased stress in chickens, decreased feed utilization efficiency, and decreased productivity (Gholami et al., 2020; Permana et al., 2020). Stress in chickens adversely affects their physiology, immunology, and microbiology, leading to decreased performance (Sugiharto et al., 2018). Research on the performance of Korean native chickens showed that body weight increased significantly with decreasing density, and feed consumption decreased significantly with increasing density (Kim et al., 2020). HSD can damage the microflora in the gut, which is bad for gut health because it leads to inflammation and changes in the microbe population, throwing off the balance of the gut (Sugiharto, 2022). In addition, HSD also affects blood biochemical parameters, including a decrease in lymphocytes, an increase in heterophils with an increase in the ratio of heterophils to lymphocytes, a decrease in the weight of the bursa fabricius, an increase in malondialdehyde (MDA) levels in liver tissue and a decrease in antibody titers against Newcastle Disease Virus (NDV) and avian influenza viruses (Astaneh et al., 2018; Li et al., 2019; Meena et al., 2022).

Providing low levels of crude protein in poultry diets is an effective strategy for reducing feed costs, ammonia emissions from poultry waste, and environmental impacts (Abd-Elsamee et al, 2020). However, low crude protein diets have negative impacts on growth performance, changes in gut morphology, the immune system, and physiological and biochemical indices in broiler chickens (Lee et al., 2016; Amiri et al., 2019; Kamely et al., 2020).

One of the local chicken breeds in Indonesia that can be developed to meet the increasing demand for chicken meat is Sentul Selection (SenSi) 1 Agrinak Chicken. SenSi 1 Agrinak chicken has several advantages, including a 70-day live body weight of 1, 066 g/bird for males and 745 g/bird for females, feed conversion ratio (FCR) of 2.7-3.7, and relatively resistance to disease (Hasnelly et al., 2018). This study aimed to investigate the impact of stocking density and crude protein levels on the performance of SenSi 1 Agrinak chickens. The combination of stocking density and crude protein levels has never been studied before. The point of this study was to find out how stocking density and crude protein levels affect the blood parameters, bacterial populations, organ immunity, antioxidant status, and performance of SenSi 1 Agrinak chickens. The information from this study is expected to enrich the literature for further research and become a recommendation for local chicken producers.

MATERIALS AND METHODS

Experimental Design and Diets

This study was conducted in accordance with the Animal Ethics Guidelines of the Ethics Review Commission of the Faculty of Animal Husbandry and Agriculture of Diponegoro University (No. 59-05/A-11/KEP-FPP). This study used 504 local Indonesian chick, Sentul Selection (SenSi) 1 Agrinak, reared for 70 days. In the starter phase, Day Old Chicken (DOC) was kept together in stage cages and fed with the nutrient content of Metabolic Energy (ME) 2986.79 kcal/kg, Crude Protein (CP) 20%, Crude Fat 4%, Crude Fiber 5%, Calcium (Ca) 0.8-1.10%, and Phosphorus (P) 0.50% for three weeks. At week 4, the DOC were adapted to the feeding treatment.

The research treatment began at week 5 using a 3×3 factorial pattern group randomized design, repeated four times with body weight groups as replicates, resulting in a total of 36 experimental units. The treatment consisted of two factors. The first factor was cage density (SD) consisting of three treatment levels, 10 birds/m^2 (LSD), 14 birds/m^2 (MSD), and 18 birds/m^2 (HSD). The second factor was the crude protein (CP) level, which consists of two treatment levels: 14% (LCP), 16% (MCP), and 18% (HCP). The treatments were as follows: LSDLCP = 10 birds/m^2 with 14% crude protein, LSDMCP = 10 birds/m^2 with 16% crude protein, LSDHCP = 10 birds/m^2 with 18% crude protein, 14 birds/m^2 with 14% crude protein, MSDMCP = 14 birds/m^2 with 16% crude protein, MSDHCP = 14 birds/m^2 with 18% crude protein, HSDLCP = 18 birds/m^2 with 14% crude protein, HSDMCP = 18 birds/m^2 with 16% crude protein, HSDHCP = 18 birds/m^2 with 18% crude protein.

SenSi 1 Agrinak chickens were olaced in a 1 × 1 × 70 m stage cage per treatment. Feed was provided ad libitum until the end of the rearing period. Nutrient content and crude protein composition are shown in Tables 1 and 2. Chickens were vaccinated Newcastle disease (ND) and infectious bronchitis (IBD) by applying one eye drop at 3, 18, and 39 days of age. The Gumboro vaccine (Cevac Transmune IBD®, Ceva Animal Health, Indonesia) was administered in the drinking water at 11 and 24 days of age.

Data Collection

Blood Sample: On the 63th day, two birds from each experimental unit were selected for blood sampling. Serum complete blood count was performed. Blood was drawn from the chick wing vein and placed it in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) and a vacutainer without anticoagulant, then processed the blood accordingly. Blood in the latter vacutainer was allowed to clot at room temperature and then centrifuged at 5,000 rpm for 15 min to produce serum. Serum was frozen until

Table 1: Nutrient content of ration ingredients in dry matter

Feed Ingredients	**ME	*Crude Protein	*Crude Fat	*Crude Fiber	*Ca	*p
Rice bran	2462,73	8.78	5.71	11.19	0.04	1.40
DDGS	2697,31	20.91	3.91	10.32	0.05	0.00
Yellow corn	3123,12	6.82	2.01	2.01	0.02	0.30
Cassava flour	2681,44	2.58	1.71	12.84	0.30	0.35
Wheat Bran	2935,66	15.14	2.87	3.61	0.14	1.10
MBM	2103,42	44.00	470	14.90	11.00	3.00
Fish meal	2632,71	52.95	11.67	4.46	0.50	2.60

Source : * Results of proximate analysis of Laboratory Assessment Institute for Agricultural Technology of Central Java (2022)
** The results of calculations using the formula Bolton cit Sugiharto et al., (2018)
ME, Metabolizable energy

Table 2: Composition and nutritional content of research rations

Feed Ingredients	Composition (%) LCP	Composition (%) MCP	Composition (%) HCP
Rice bran	12.00	10.00	5.00
DDGS	24.00	24.00	28.00
Yellow corn	2800	27.50	24.00
Cassava flour	12.21	10.21	6.21
Wheat Bran	9.00	10.00	15.50
MBM	4.00	6.00	8.00
Fish meal	6.00	7.50	8.50
Mineral	300	3.00	3.00
DL-Methionine	0.25	0.25	0.25
Tryptophan	0.27	0.27	0.27
Threonine	077	0.77	0.77
Total	100.00	100.00	100.00
Nutritional Content:			
Metabolic Energy (kcal/kg)	2632,33	2605,66	2603,97
Crude protein (%)	14.44	16.05	18.00
Crude fat (%)	3.58	3.70	3.79
Crude Fiber (%)	7.15	7.28	7.10
Calcium (Ca) (%)	1.63	2.06	2.28
Phosphorus (P) (%)	0.83	0.95	0.96

Calculation of the feed based on the results of the proximate analysis of the Laboratory of Assessment Institute for Agricultural Technology of Central Java (2022)
LCP, Low Crude Protein, MCP, Medium Crude Protein, HCP, High Crude Protein, DDGS, Distiller's Dried Grain with Solubles; MBM, Meat and Bone Meal

analysis (Yudiarti et al., 2020). Hematology analyzers were used to measure and determine the total blood count (Semarang Animal Health Laboratory).

Microbial population: Microbial populations were observed in slaughtered chickens aged 70 days, which were collected from 2 chickens from each experimental unit. The digestive tract was obtained by slaughtering the chickens and performing a chest dissection to remove it. The small intestine (ileum and cecum) was separated for digestive samples. Then the digestive samples placed into a vial and store it in an ice bag containing ice cubes to preserve the sample. Coliform bacteria were counted as red colonies on MacConkey agar (Merck KGaA) after 24 h of aerobic incubation at 38°C, whereas lactic acid bacteria (LAB) were counted on de Man, Rogosa, and Sharpe agar (MRS; Merck KGaA) after 48 h of anaerobic incubation at 38°C.

Organ lymphoid: The relative weight of immune organs, including the bursa of Fabricius, spleen, and thymus, is determined by measuring the weight of the organ, dividing the value by the live body weight, and multiplying the re-

sult by 100% (El-deep et al., 2016).

Antioxidant Activity: Malondialdehyde activity (MDA) of each sample was measured by reactive matrix test using thiobarbituric acid (TBA). Samples were vortexed, mixed with 8.1% sodium dodecyl, and left at room temperature for 10 minutes. Controls were treated in the same way. After incubation, 20% acetic acid and 0.6% TBA were added to the samples, which were then placed in a water bath for 1 hour at 90-95°C. Subsequently, butanol: pyridine (15:1) was added to the supernatant, and the mixture was stirred and centrifuged. Malondialdehyde activity (MDA) was expressed in nmol/ml.

Antibody Titre: The hemagglutination inhibition (HI) test for ND antibodies in order to determine the antibody titre was performed according to the method by Agusetyaningsih et al. (2022). Two-fold serial dilutions of the serum samples were prepared in microtiter plates with normal saline. Next, 0.05 ml of the ND antigen was applied to each well of the plate. Three rows of wells were used as controls: the first row contained only ND antigen (positive control), and the third row contained normal saline with red blood cells. The plate was shaken using a Titertek plate shaker and maintained at room temperature for 30 minutes. then, 0.05 ml of broiler red blood cells were added to each well. The HI titre was defined as the highest dilution that could inhibit 50% agglutination.

Growth performance: To evaluate growth performance, body weight, feed consumption and feed efficiency were measured in chickens aged 29-70 days. Body weight gain was calculated by subtracting the initial body weight and the end body weight of the maintenance period. Daily feed consumption was calculated by subtracting the total feed given to the entire flock. Feed efficiency percentage was calculated by dividing weight gain by feed consumption.

Statistical Analysis

The data obtained were analyzed using the test analysis of variance (ANOVA) with a factorial Group Random Design by SPSS. The data that showed a significant effect was continued with Duncan's test to determine the differences in each treatment.

RESULTS

Blood Profiles

The Statistical analysis data for the effect of stoking densities and crude protein levels on blood profiles were presented in Table 3. It indicated no significant effect ($p > 0.05$) of the treatments applied in this study. In addition, there was no interaction between different stoking densities and crude protein levels.

Microbial Population

The number of selected bacteria and pH in the intestines of Sensi 1 Agrinak chickens were shown in Table 4. Statistical analysis showed that stocking density had a significant effect ($p < 0.05$) on the population of Lactic Acid Bacteria (LAB) and coliform in the ileum, but no significant effect on the cecum ($p > 0.05$). Data showed that LAB and coliform populations in HSD and MSD treatments were higher than those in LSD treatment. Crude protein treatment had no significant effect on LAB or coliform populations in the ileum and cecum. There was no interaction between different stocking densities and crude protein levels on LAB and coliform populations.

Immune Organs

Statistical analysis showed that different stocking densities and crude protein levels had no significant effect ($p > 0.05$) on the percentage weight of the bursa fabricius and spleen but had a significant effect ($p < 0.05$) on the percentage weight of the thymus (Table 5). The percentage of thymus weight in HSD was the same as that in MSD, but the weight was lower than that in LSD. Statistical analysis of crude protein content in the lymphoid organs (bursa fabricius, thymus, and spleen) showed no significant effect ($p > 0.05$). There was no interaction between stocking density and crude protein content.

Antioxidative Status and Antibody Titres Against Newcastle Disease (ND)

The effect of different stocking densities and crude protein levels on MDA levels and antibody titres to NDV were shown in Table 6. Statistical analysis showed no significant effect ($p > 0.05$) of stocking density treatment and crude protein content on MDA levels. There was an interaction between different stocking density and crude protein content. The interaction occurred in the treatment of LSDLCP if the increased stocking density of MSDLCP increased MDA levels, while the treatment of LSDMCP and LSDHCP if the increased stocking density of MSDMCP and MSDHCP decreased MDA levels. Statistical analysis of different stocking density treatments on NDV antibody titres showed no significant effect ($p > 0.05$). However, the treatment of different levels of crude protein showed a significant effect on the NDV antibody titre. The NDV antibody titre response in the MCP treatment was similar to the HCP treatment, but it was higher than the LCP treatment. There was no interaction between stocking density and crude protein level with NDV antibody titres.

Growth Performance

Statistical analysis of stocking density treatment in Sen-Si 1 Agrinak chickens showed a significant effect on feed consumption ($p < 0.05$), but no significant effect on body weight gain and feed efficiency (Table 7). The total feed

Table 3: Blood profile of Sensi 1 Agrinak Chicken

Treatment	Items											
	Leukocytes	Erythrocytes	Haemoglobin	Haematocrits	Thrombocytes	Lymphocytes	Heterophils	MCV	MCH	RDW-SD	RDW-CV	MPV
	(10^3/L)	(10^6 /L)	(g/dL)	(%)	(10^3/L)	(10^3/L)	(10^3 /L)	(fl)	(pg)	(fl)	(%)	(fl)
Reference Values	1.2-3.0	2.5-3.5	7.0-13.0	30.0-39.0	rare	7.0-17.5	3.0-6.0	90.0-140.0	33.0-47.0	0.1-99.0	0.1-99.0	0.1-30.0
Stoking Density												
LSD	10.87	2.61	11.46	31.56	24.00	95.44	11.23	121.89	35.28	56.38	12.80	9.48
MSD	10.39	2.64	11.40	32.19	20.21	91.73	12.25	123.00	33.73	58.78	12.66	9.18
HSD	11.16	2.64	11.06	31.98	24.08	98.33	13.31	122.27	30.15	57.86	12.10	9.26
Crude protein (%)												
CP14	10.41	2.68	10.88	32.33	24.58	90.67	11.42	121.88	29,57	56.99	11.95	9.37
CP16	11.52	2.58	11.67	31.27	21.08	101.90	13.38	122.19	35,64	57.16	12.96	9.15
CP18	10.49	2.63	11.38	32.13	22.63	92.94	12.00	123.10	33,94	58.87	12.66	9.40
SEM	0.54	0.10	0.29	1.33	2.67	4.80	0.81	0.78	1.46	1.92	0.37	0.24
Stoking Density * Crude Protein												
LSDCP14	9.26	2.67	10.69	32.06	24.50	78.63	7.75	121.44	31.26	54.80	11.91	9.24
LSDCP16	12.50	2.55	12.00	30.56	20.63	110.94	14.06	121.20	39.56	52.29	13.15	9.65
LSDCP18	10.86	2.62	11.69	32.06	26.88	96.75	11.88	123.03	34.98	62.04	13.34	9.55
MSDCP14	11.22	2.76	11.13	33.25	24.38	98.44	13.81	121.76	31.78	58.31	12.68	9.51
MSDCP16	10.11	2.57	11.88	31.50	18.50	89.75	11.44	122.81	35.85	60.63	13.09	9.41
MSDCP18	9.85	2.58	11.19	31.81	17.75	87.00	11.50	124.44	33.55	57.40	12.21	9.63
HSDCP14	10.76	2.62	10.81	31.69	24.88	94.94	12.69	122.43	25.65	57.85	11.25	9.36
HSDCP16	11.96	2.61	11.13	31.75	24.13	105.00	14.63	122.56	31.50	58.56	12.64	9.40
HSDCP18	10.76	2.68	11.25	32.50	23.25	95.06	12.63	121.83	33.30	57.16	12.43	9.01
SEM	0.94	0.18	0.50	2.31	4.62	8.31	1.40	1.36	2.52	3.33	0.64	0.41
P-value												
Stoking Density	0.61	0.98	0.58	0.94	0.51	0.63	0.21	0.60	0.18	0.68	0.38	0.66
Crude protein (%)	0.29	0.78	0.16	0.84	0.65	0.24	0.23	0.53	0.09	0.75	0.16	0.73
Stoking Density * Crude Protein	0.26	0.97	0.78	0.99	0.85	0.20	0.06	0.75	0.84	0.36	0.62	0.24

a,b,c, Means on the same row with different superscripts are significantly different, **=p<0.01, *= p<0.05
LSD, Low Stoking Density 10 bird / m^2; MSD, Medium Stoking Density 14 bird / m^2; HSD, High Stoking Density 18 bird / m^2; CP14, crude protein 14%; CP16, crude protein 16%; CP18, crude protein 18%;
SEM, Standard error of means; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW-SD, red blood cell distribution width-standard deviation; RDW-CV, red blood cell distribution width-coefficient variation; MPV, mean platelet volume
Reference values: Weiss et al., 2010

Table 4: Selected bacteria population in the intestine of Sensi 1 Agrinak Chicken

Treatment	Items			
	Lactic Acid Bacteria		Coliform	
	Ileum	Secum	Ileum	Secum
	log cfu/g			
Stoking Density				
LSD	5.60[b]	7.66	5.35[b]	6.26
MSD	6.26[a]	7.59	6.10[a]	6.33
HSD	6.15[a]	7.82	6.28[a]	6.42
Crude protein (%)				
CP14	5.97	7.61	6.03	6.27
CP16	6.16	7.71	6.01	6.37
CP18	5.88	7.74	5.71	6.37
SEM	0.18	0.10	0.15	0.10
Stoking Density * Crude Protein				
LSDCP14	5.41	7.51	5.20	6.38
LSDCP16	5.76	7.91	5.77	5.98

LSDCP18	5.60	7.57	5.09	6.43
MSDCP14	6.65	7.66	6.32	6.14
MSDCP16	6.21	7.45	5.87	6.62
MSDCP18	5.91	7.67	6.13	6.23
HSDCP14	5.86	7.68	6.56	6.30
HSDCP16	6.47	7.78	6.38	6.50
HSDCP18	6.11	7.99	5.91	6.45
SEM	0.31	0.17	0.25	0.18
P-value				
Stoking Density	0.04*	0.26	0.00**	0.58
Crude protein (%)	0.54	0.63	0.25	0.76
Stoking Density * Crude Protein	0.39	0.30	0.23	0.12

a,b,c, Means on the same row with different superscripts are significantly different, **=p<0.01, *= p<0.05
LSD, Low Stoking Density 10 bird / m^2; MSD, Medium Stoking Density 14 bird / m^2; HSD, High Stoking Density 18 bird / m^2; CP14, crude protein 14%; CP16, crude protein 16%; CP18, crude protein 18%;
SEM, Standard error of means

Table 5: Immune organs of Sensi 1 Agrinak Chiken

Treatment	**Items**		
	Bursa Fabricius	**Thymus**	**Spleen**
	% final body weight		
Stoking Density			
LSD	0.39	0.67[a]	0.36
MSD	0.31	0.53[b]	0.46
HSD	0.32	0.53[b]	0.40
Crude protein (%)			
CP14	0.33	0.64	0.43
CP16	0.32	0.53	0.36
CP18	0.37	0.57	0.43
SEM	0.03	0.04	0.04
Stoking Density * Crude Protein			
LSDCP14	0.41	0.75	0.37
LSDCP16	0.39	0.60	0.31
LSDCP18	0.37	0.67	0.41
MSDCP14	0.26	0.59	0.53
MSDCP16	0.27	0.51	0.36
MSDCP18	0.39	0.49	0.48
HSDCP14	0.26	0.57	0.40
HSDCP16	0.27	0.48	0.41
HSDCP18	0.39	0.54	0.39
SEM	0.04	0.08	0.07
P-value			
Stoking Density	0.07	0.05*	0.24
Crude protein (%)	0.43	0.22	0.33
Stoking Density * Crude Protein	0.36	0.95	0.68

a,b,c, Means on the same row with different superscripts are significantly different, **=p<0.01, *= p<0.05
A1, stoking density 10 bird / m^2; A2, stoking density 14 bird / m^2; A3, stoking density 18 bird / m^2;
B1, crude protein 14%; B2, crude protein 16%; A3, crude protein 18%; SEM, Standard error of means

Table 6: Serum malondialdehyde (MDA) levels and Titers antibodies against Newcastle disease virus (NDV) of Sensi 1 Agrinak Chicken

Treatment	Items	
	MDA	Antibody Titer
	(nanomolar/ml)	log_{10}
Stoking Density		
LSD	3.89	2.67
MSD	3.67	2.58
HSD	4.24	2.92
Crude protein (%)		
CP14	4.02	2.17^{b}
CP16	3.88	3.42^{a}
CP18	3.90	2.58^{ab}
SEM	0.91	0.34
Stoking Density * Crude Protein		
LSDCP14	3.12^{bc}	1.25
LSDCP16	3.95^{abc}	4.25
LSDCP18	4.59^{a}	2.50
MSDCP14	4.66^{a}	2.75
MSDCP16	3.50^{abc}	2.75
MSDCP18	2.86^{c}	2.24
HSDCP14	4.29^{ab}	2.50
HSDCP16	4.20^{ab}	3.25
HSDCP18	4.24^{ab}	3.00
SEM	1.61	0.60
P-value		
Stoking Density	0.32	0.78
Crude protein (%)	0.92	0.05*
Stoking Density * Crude Protein	0,03	0.16

a,b,c, Means on the same row with different superscripts are significantly different, **=p<0.01, *= p<0.05
A1, stoking density 10 bird / m^2; A2, stoking density 14 bird / m^2; A3, stoking density 18 bird / m^2;
B1, crude protein 14%; B2, crude protein 16%; A3, crude protein 18%;
SEM, Standard error of means

Table 7: Performance of Sensi 1 Agrinak Chicken

Treatment	Items		
	Feed Intake	Body Weight Gain	Feed Efficiency
	(g/bird)	(g/bird)	%
Stoking Density			
LSD	588.95^{a}	657.45	29.62
MSD	559.26^{ab}	643.26	27.88
HSD	530.02^{b}	618.38	27.49
Crude protein (%)			
CP14	542.7	610.01	27.75
CP16	564.13	653.09	28.91
CP18	571.4	655.99	28.32
SEM	12.55	22.47	0.93
Stoking Density * Crude Protein			

LSDCP14	2137.91	652.80	30.91
LSDCP16	2174.85	625.77	28.99
LSDCP18	2382.59	693.78	29.15
MSDCP14	2211.42	555.52	24.99
MSDCP16	2391.45	696.82	29.14
MSDCP18	2312.81	677.44	29.5
HSDCP14	2277.17	621.70	27.53
HSDCP16	2258.6	636.68	28.6
HSDCP18	2289.12	596.75	26.32
SEM	43.27	38.91	1.61
P-value			
Stoking Density	0.01	0.47	0.24
Crude protein (%)	0.26	0.29	0.68
Stoking Density * Crude Protein	0.36	0.16	0.25

a,b,c, Means on the same row with different superscripts are significantly different, **=p<0.01, *= p<0.05
A1, stoking density 10 bird / m^2; A2, stoking density 14 bird / m^2; A3, stoking density 18 bird / m^2;
B1, crude protein 14%; B2, crude protein 16%; A3, crude protein 18%;
SEM, Standard error of means

consumption in LSD was similar to that in MSD, but higher than that in HSD. Crude protein treatment had no effect (p > 0.05) on feed consumption, body weight gain, and feed efficiency. There was no interaction between stocking density and crude protein level on chicken performance.

DISCUSSION

Evaluation of long-term stress and immune responses in poultry to stressors can be measured through blood biochemical and hematological parameters, leukocyte differentiation, and phagocytosis activity (Saeed et al., 2019). The bodies of chickens that are stressed by heat show changes in circulating leukocyte counts, with a lot more heterophiles and fewer lymphocytes (Bin-Jumah et al., 2020). This study showed no effect of stocking density on blood profile. However, based on the average threshold value of the blood profile, according to Weiss (2010), leukocyte and heterophile values were above the average threshold, meaning that all stocking density treatments indicated stress. The results of this study are in line with the findings of Attia et al. (2020) and Sapsuha et al. (2022), who reported that the blood profile of broilers with high stocking density did not differ from the blood profile of broilers with average stocking density. In contrast, Nasr et al. (2021) discovered that the number of chickens in a flock affected the biochemical parameters of their blood. When the number of chickens in a flock was higher, the number of heterophiles in the blood was higher, and the number of lymphocytes was lower. This study showed no significant effect of different crude protein levels on the blood profile. Amiri et al. (2019) reported no significant differences in lymphocytes, heterophils, monocytes, eosinophils, and basophils. The dietary crude protein (CP) content associated with heat stress (HS) lies in its contribution to catabolism, which triggers higher metabolic heat production than fats and carbohydrates in the body.

Microbial populations in chickens are influenced by several factors, such as chicken strain, sex, and environment (Carrasco et al., 2019; Yadav et al, 2019). High stocking density conditions in broiler chickens disrupt microbial ecology in the intestine, disrupting intestinal barrier function potentially reducing productive performance and increasing health problems for poultry (Goo et al., 2017). The study by Li et al. (2022) reported that an increase in chicken population density significantly changed the composition of the microbiota in the ileum. The relative abundance of Lactobacillales, such as Lactobacillus, Enterococcus, and Streptococcus, decreased markedly in higher-density broilers (15.5 birds/m^2) compared to those with lower densities (12.5 birds/m^2). The results of this study show that stocking density has a significant effect on LAB and coliform populations in the ileum. LAB and coliform populations in MSD and HSD treatment have more significant numbers than in LSD treatment. The results showed that the higher the stocking density, the higher the LAB and coliform populations. The results of this study are different from those reported by Özcan et al. (2015), which stated that the population of lactobacilli in the ileum with high stocking density (20 birds/m^2) is lower than the population of lactobacilli in the ileum with low stocking density (10 birds/m^2).

The results of this study suggest that the different pro-

tein-level treatments did not affect LAB and coliform populations in the ileum and cecum. The study results are consistent with the findings (Abd-Elsamee et al., 2020), which reported no effect of variations in crude protein levels on the LAB population. Conversely, Cesare et al. (2019) reported that a decrease in crude protein levels negatively affected the LAB population, with lower protein levels resulting in a decrease in the LAB population.

Poultry have immune organs consisting of the thymus, spleen, and bursa fabricius. The immune organs produce an immune response that produces various cytokines and chemokines to increase the immune response to protect the body from various diseases and stresses due to environmental stress that can cause death (Ruo-Han, 2023). The results of this study show that stocking density has a significant effect on the percentage of lymphoid organs (thymus weight). As the study showed Li et al. (2019), the percentage of bursa fabricius weight that changed between high stocking density (18 birds/m^2) and low stocking density (15 birds/m^2) in male Arbor Acres broilers was significantly affected by stocking density. The higher the stocking density, the smaller the exchange weight of bursa fabricius. Yanai et al. (2018) stated that high stocking density harms lymphoid organs, which has the potential to cause a low immune response. The thymus, bursa fabricius, and spleen become more active when antigens come in the form of sheep red blood cells. This leads to more excellent lymphocyte production and can change those organs. The results of this study showed that there is no effect of crude protein levels on lymphoid organs. The results of the same study reported by Kamely et al. (2020) did not find a significant effect on lymphoid organs in broiler chickens with crude protein levels of 21.23% and crude protein of 23.78%.

Changes in the maintenance system, stocking density, farm location, and other physical environments can lead to physiological stress and impair the immune response (Kamal et al., 2018). Selvam et al. (2017) reported that broilers can experience oxidative stress when they are crowded, which can lead to lower levels of glutathione (GSH) and higher levels of malondialdehyde (MDA) in their liver tissue. The current study's findings are consistent with those reported by Ha et al. (2021) in native Korean broiler chickens, as different stocking densities do not influence MDA activity. However, this differs from the results of a previous study by Gao et al. (2023), which reported that different stocking densities influence MDA activity in broiler chickens; the higher the stocking density, the higher the MDA content. Food proteins have a more significant thermogenic effect than carbohydrates, and a high-protein diet lowers body temperature and mortality but does not improve broiler growth (Teyssier et al., 2022).

The results showed that different stocking densities did not affect NDV titres. In contrast to the study conducted by Houshmand et al. (2012), that the density of the higher the meal, the NDV titres value is smaller than the average density. High stocking density capacity causes an increase in the temperature in the cage, which eventually causes heat stress. A decrease in antibodies during heat stress causes an increase in NDV, infectious bursal disease virus (IBDV), and infectious bronchitis disease virus (IBV) (Vandana et al., 2021). According to Ezzulddin et al. (2022), antibody values can be influenced by intrinsic and extrinsic factors, including environmental factors, chicken-rearing management factors (vaccine programs), and chicken genetics factors that significantly affect the immune system. In this study, different crude protein levels significantly influence NDV titres. Same with Sigolo et al. (2014) who reported a significant impact of crude protein levels on NDV titres in broiler chickens. Specifically, higher crude protein levels resulted in higher NDV titres after the third injection. However, this contrasts with the study by Houshmand et al. (2012), which reported that there was no effect of different crude protein levels (CP 17% and CP 20%) on broiler chicken NDV titres.

Environmental temperature plays a vital role in the growth and consumption of feed. Cold environmental conditions lead to increased feed consumption as the bird's body requires more energy for warmth. Increasing the stocking density reduces the energy used to heat the bird's body, allowing most of the energy from the feed to be used for growth (Gholami et al., 2020). According to Özcan et al. (2015), chickens raised with LSD treatment had greater access to food and water compared to those treated with HSD, resulting in limited movement in their cages. Reduced feed consumption and resulted in weight loss. The study found that stocking density affects feed consumption. Higher stocking densities resulted in lower feed consumption, but the study by Son et al. (2022) found no significant effect on feed consumption at stocking densities of 16, 18, 21, 23, and 26 birds/m^2. The analysis revealed no significant effect of stocking density on feed efficiency or body weight gain. However, Gholami et al. (2020) reported that higher stocking densities resulted in lower weight gain in broiler chickens. This study found no effect of differences in crude protein levels on the performance of SenSi-1 Agrinak chickens, and the same study by Gervais et al. (2019) in broiler chickens also found no effect of different levels of crude protein on feed consumption and weight gain. Law et al. (2018) found that differences in crude protein levels impacted feed consumption and weight gain in chickens aged 28 to 35 days.

The study's flaw is that all LSD, MSD, and HSD treatments suggest that SenSi 1Agrinak chicken are stressed

because leukocyte and heterophile values are over the average threshold. It is required to carry out more thorough research on blood sampling techniques that are seted to the amount of stocking density, method, and time of periodic blood sample because it is not certain that these factors caused the stress. Additionally, more investigation is required to validate the higher coliform populations at high stocking densities accompanied by a higher LAB population.

CONCLUSIONS

High stocking density and decreased crude protein levels have the potential to interfere with the health of SenSi 1 Agrinak chickens, but do not affect growth performance. The results of this study can provide information about optimal stocking density and efficient crude protein levels on the health and performance of SenSi 1 Agrinak chickens.

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CONFLICT OF INTEREST

The authors declare no conflict related to the material described in the manuscript.

NOVELTY STATEMENT

The novelty of this study is information that stocking density and crude protein levels affect blood parameters, bacterial population, organ immunity, antioxidant status, and performance of SenSi 1 Agrinak chickens.

AUTHORS CONTRIBUTIONS

F.R.P. Hantoro compiled the research concept, collected and analyzed processed research data, and drafted the manuscript. R. Hayati collected the data. D. Sunarti, T. Yudiarti, and S. Sumarsih supervised and finalized the manuscript.

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