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Research Article

Growth and Haematological Response of Broilers Raised at High-Density Pens and Fed Germinated Papaya Seed and Chitosan Blend

Sugiharto Sugiharto*, Ikania Agusetyaningsih, Endang Widiastuti, Hanny Indrat Wahyuni, Turrini Yudiarti, Tri Agus Sartono

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java Province, Indonesia.

Abstract | The study investigated the effects of germinated papaya seed and a mixture of germinated papaya seed and chitosan on growth and haematological parameters in broilers stocked in high-density pens from 15 to 28 days old. A total of 490 broiler chicks were distributed into four groups, including CONT (chicks receiving basal feed and raised under normal density [10 chicks per m^2]), HSD (chicks receiving basal feed and raised under high density [20 chicks per m^2]), HSD-P (chicks receiving 0.5% germinated papaya seed flour and raised under high density) and HSD-PC (chicks receiving 0.5% germinated papaya seed flour and 0.2% chitosan, and raised in high-density condition). Growth and feed intake were measured weekly, while blood was collected on day 28. Despite being lower ($P < 0.05$) than CONT, HSD-P and HSD-PC chickens consumed more ($P < 0.05$) feed than HSD chickens. Body weight and weight gain were lower ($P < 0.05$) in HSD compared to the CONT, HSD-P and HSD-PC groups. The European Production Higher Efficiency Factor (EPEF) tended ($P = 0.06$) to be higher in HSD-PC than in HSD chicks. While there was no significant effect of the treatments on complete blood counts and serum biochemical parameters, serum creatinine concentration was higher ($P < 0.05$) in HSD and HSD-P than in CONT and HSD-PC. In conclusion, germinated papaya seeds or a mixture of germinated papaya seeds and chitosan improved broiler production performance and kidney health while alleviating muscle protein catabolism in high-density pens from days 15 to 28.

Keywords | Broiler, Chitosan, Germinated seed, High stocking density, Stress

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***Correspondence** | Sugiharto Sugiharto, Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java Province, Indonesia; **Email:** sgh_undip@yahoo.co.id
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INTRODUCTION

To maximize the potential of broiler house, farmers typically raise broiler chickens in high numbers when the chickens are young or still have low body weight. As the age and body weight of the chickens increase, the farmers usually do thinning (partial depopulation or harvest some chickens early to reduce the number of chickens in cage), so that the number of chickens raised per square meter is not too high. In most commercial broiler farms, it is common to practice thinning starting on the 28th day of rearing. Indeed, reducing the number of chickens per square meter is vital to give more space or room for other chickens to grow according to the target weight at the end of the harvest (Marlina et al., 2015). Also, reducing the number of chickens per square meter is crucial to avoid stress due to high stocking densities and improve the welfare of chickens. In terms of production, stress caused by a high number of chickens per square meter can greatly compromise broiler's production performance and health condition (Sugiharto, 2022). So far, studies on the negative impact of high stocking density on the productivity and physiological condition of broiler chickens approaching harvest time (aged 35 days and over) have been widely carried out (Pawesti et al., 2022; Sugiharto et al., 2022a). However, studies on the negative consequences of high density on chickens in the period before thinning (before 28 days of age) are scarce in the literature. Indeed, data on the impact of high stocking density on broiler chickens aged 15 to 28 days are required to address whether high stocking density can negatively impact on the growth rate and physiological condition of broiler chickens at an early age.

Thinning as early as possible is the best way to prevent chickens from high stocking density's negative effects. This is because as the stocking density (number of chicks or total body weight per square meter of housing area) increases with age and body weight, competition for feed and drinking water among chickens becomes more intense. A high stocking density, particularly for broiler chickens raised in a deep litter system, can increase ammonia levels in the litter while also making the litter wetter (Theresah et al., 2020). However, early thinning may result in inefficient use of broiler houses. To maximize cage efficiency as well as alleviate chickens from the negative impacts of high stocking density, the use of additives or supplements in feeds or drink is encouraged (Sugiharto, 2022). To deal with the detrimental effect of stress in broiler production, natural ingredients are increasingly being used as feed additives (Pawesti et al., 2022). In contrast to synthetic materials, which can leave residues on broiler meat, natural ingredients do not leave residues, making broiler chicken products safe for consumers (Sugiharto, 2022). One of the natural components that show the potential to improve broilers' physiological conditions and health is papaya seed, which is a good source of antioxidants and an antibacterial agent (Ying et al., 2021). Yet, the high fibre content and the presence of some antinutrient components in papaya seed (such as phytate, saponin, oxalate, etc.) may hinder its utilization as a feed additive or supplement for broiler chickens. A very recent study reported that compared to non-germinated, germinated papaya seeds showed greater antioxidant capacity and lower fibre content (Sugiharto et al., 2022b), so it can provide more benefits for broiler chickens. Chitosan, an amino polysaccharide derived from the deacetylation of crustacean and insect chitin, is another natural component that could be used as a stress-relieving supplement. A study by Koochacksaraei et al. (2020) revealed chitosan could improve the growth performance and antioxidant status of stressed-broilers. Likewise, Chang et al. (2020) found a positive effect of chitosan in improving the growth performance and oxidative status of heat-stressed broiler chickens. Several other studies also found that chitosan increased liver metabolic activity in ruminants (Mushawwir et al., 2020), improved liver function and reduced liver inflammation in broiler (Mushawwir et al., 2021), and native chicken (Hanifah et al., 2022), and effectively prevents lipogenesis in native chicken (Kharazi et al., 2022).

Combining two or more bioactive substances has frequently been done to create a synergistic effect and increase the host's benefit. Al-Baqami and Hamza (2020) combined chitosan and vanillin and found a synergistic effect between these ingredients. Indeed, the combination of chitosan and vanillin demonstrated more antioxidant activity than either compound alone. Likewise, Rezazadeh et al. (2020) reported the synergistic activity between chitosan and brown marine algae extract in enhancing the antimicrobial activity of algae. So far, no published data reveals the use of germinated papaya seed flour and a mixture of germinated papaya seed flour and chitosan on stressed broilers. This study, therefore, aimed to investigate the effects of germinated papaya seed and a mixture of germinated papaya seed and chitosan on growth and haematological parameters in broilers stocked in high density pens from 15 to 28 days old.

MATERIALS AND METHODS

Ethical approval

The Animal Ethical Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang approved the *in vivo* study with the approval number 58-04b/A-5/KEP-FPP.

Treatments preparations

The germinated papaya seed used in this study was prepared according to the method as described by Sugiharto et al. (2022b). In brief, the ripe papaya seeds were collected from street vendors around the university. The seeds were washed with running water and aerated on a tray at room temperature for a day (24 hours). The seeds were then soaked for 24 hours, and put into a bucket that has been perforated. The seeds were allowed to germinate for two weeks. To keep the seeds moist, the germinated seeds were daily sprayed with water. After germination, the seeds were sun-dried, and finely ground into powder. The germinated papaya seed flour was placed in room temperature until use. The chitosan used in this study was a commercial chitosan (CV. ChiMultiguna, Indonesia) produced from shrimp shells with a particle size of 200 nm.

In vivo experiment

A total of 490 Cobb (unsex) broiler chicks were used in the study, which was set up based on a completely randomized design. During their first 14 days of rearing, the chicks were communally raised on commercial prestarter feed, which, according to the feed label, contained 22-24% crude protein, no more than 5% crude fibre, 5% crude fat, and 7% ash. On day 15, the chicks were randomly distributed into 4 treatment groups with 7 replications/pens. The treatment groups included CONT (chicks receiving basal feed and raised under normal density conditions [10 chicks per m^2]), HSD (chicks receiving basal feed and raised under high density condition [20 chicks per m^2]), HSD-P (chicks receiving a feed containing 0.5% germinated papaya seed flour and raised under high density conditions) and HSD-PC (chicks receiving a feed containing 0.5% germinated papaya seed flour and 0.2% chitosan, and raised in high density condition).

The chicks were raised in an open-sided broiler house with beds made of rice husks. A steady lighting schedule was used throughout the study. According to the Indonesian National Standard for broiler finisher feed, the feeds (in mash form) were formulated (Table 1). The chicks were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB) on day 0. At the age of 18, the chicks also received the ND immunization.

Table 1: Feed compositions of broilers day 15-28

Items	%, unless otherwise noted
Yellow maize	58.5
Palm oil	2.96
Soybean meal	34.7
DL-methionine	0.19
Bentonite	0.75
Limestone	0.75
Monocalcium phosphate	1.30
Premix[1]	0.34
Chlorine chloride	0.07
Salt	0.40
Chemical compositions	
ME (kcal/kg)[2]	3,000
Crude protein, %	20.0
Crude fibre, %	5.51
Ca, %	1.02
P (available), %	0.58

[1]Per kg of feed contained 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vit A, 250,000 IU vit D_3, 1,350 g pantothenic acid, 1,875 g vit E, 250 g vit K_3, 250 g vit B_1, 750 g vit B_2, 500 g vit B_6, 2,500 mg vit B_{12}, 5,000 g niacin, 125 g folic acid and 2,500 mg biotin

[2]ME (metabolizable energy) was calculated based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

Sample collections and analysis

On days 21 and 28, feed intake and body weight were measured. Blood was drawn from the wing vein of one female chick (one per pen; seven per treatment group) on day 28. For complete blood count analysis, blood was placed in a vacutainer with ethylenediaminetetraacetic acid (EDTA), and the remaining blood was placed in a vacutainer without an anticoagulant to create serum. According to the manufacturer's description, the Prima Fully-Auto Hematology Analyzer (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) was used to measure the complete blood counts. The coagulated blood was let at room temperature for around 2 hours and centrifuged for 10 minutes at 5,000 rpm to produce serum. The serum was kept in a freezer (at -10°C) pending analysis. On the basis of enzyme-based colorimetric techniques, the lipid profile (total triglycerides, total cholesterol, low-density lipoprotein and high-density lipoprotein) and the levels of uric acid and creatinine in serum were determined. The concentrations of total protein, albumin, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in broiler chicken serum were measured using spectrophotometric/photometric methods. The albumin value in serum was deducted from the total protein value to get the data for globulin concentration. Serum samples underwent all biochemical analyses in accordance with the manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany).

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA, SPSS version 16.0). Duncan's multiple analysis was performed when significant effects ($P<0.05$) of the treatments were found.

RESULTS AND DISCUSSION

The data on the production parameters of broilers from day 15 to 28 are presented in Table 2. Despite the efficiency of using the broiler house, rearing broilers in high-density conditions has been proven to impair the production performance of broilers (Sugiharto, 2022), which was also supported by the results of the current study. At a younger age (before 21 days of age), high density of deep litter broiler house had no significant impact on growth (although it reduced feed intake), but with increasing age (days 22-28), high density decreased ($P < 0.05$) growth rate of broiler chickens. In addition to the environmental stress caused by increased ammonia in the cage and poor air circulation, the larger the body of the chicken, the narrower the space for movement, making access to the feeder and drinker

Table 2: Production parameters of broiler chickens

Items	CONT	HSD	HSD-P	HSD-PC	SEM	P value
Day 14						
BW (g/chick)	357	358	356	357	0.84	0.79
Day 15-21						
BW (g/ chick)	749	720	747	740	5.00	0.15
BWG (g/ chick)	392	362	391	383	5.17	0.14
FI (g/ chick)	668[a]	578[b]	615[b]	621[b]	9.04	<0.01
FCR	1.71	1.61	1.58	1.63	0.03	0.50
EPEF	333	328	356	338	8.91	0.72
Day 22-28						
BW (g/ chick)	1217[a]	1147[b]	1210[a]	1243[a]	9.97	<0.01
BWG (g/ chick)	468[ab]	427[b]	463[ab]	503[a]	8.40	0.01
FI (g/ chick)	753[a]	668[b]	732[a]	728[a]	9.35	<0.01
FCR	1.62	1.57	1.58	1.45	0.02	0.07
EPEF	419[b]	392[b]	420[b]	498[a]	13.3	0.02
Day 15-28						
BW (g/ chick)	1217[a]	1147[b]	1210[a]	1243[a]	9.97	<0.01
BWG (g/ chick)	860[a]	789[b]	855[a]	886[a]	10.1	<0.02
FI (g/ chick)	1421[a]	1246[c]	1347[b]	1349[b]	16.6	<0.01
FCR	1.66	1.58	1.57	1.52	0.02	0.19
EPEF	375	358	387	416	7.92	0.06

[a,b,c]Means within the same row having different superscripts vary significantly ($P < 0.05$)

BW: body weight, BWG: body weight gain (body weight at weighing minus body weight at previous weighing), FI: feed intake (amount of feed given minus the rest of the feed), FCR: feed conversion ratio (ratio between feed intake and weight gain at a certain period), EPEF: European Production Efficiency Factor (average grams BWG per day × % survival rate)/(FCR × 10), CONT: chicks receiving basal feed and raised under normal density condition (10 chicks per m^2), HSD: chicks receiving basal feed and raised under high-density condition (20 chicks per m^2), HSD-P: chicks receiving a feed containing 0.5% germinated papaya seed flour and raised under high-density conditions, HSD-PC: chicks receiving a feed containing 0.5% germinated papaya seed flour and 0.2% chitosan, and raised in high-density condition, SEM: standard error of the means

Table 3: Complete blood counts of broiler chickens

Items	CONT	HSD	HSD-P	HSD-PC	SEM	P value
Erythrocytes (10^{12}/L)	2.61	2.61	2.74	2.66	0.07	0.91
Haemoglobin (g/dL)	9.93	10.1	10.9	9.93	0.20	0.32
Haematocrits (%)	31.1	31.2	32.4	32.0	0.81	0.93
MCV (fl)	120	120	119	121	0.45	0.84
MCH (pg)	38.1	38.4	40.9	37.4	0.88	0.55
MCHC (g/dL)	32.0	32.2	34.4	31.2	0.73	0.46
RDW-SD (fl)	48.9	49.1	50.5	50.4	0.77	0.84
RDW-CV (%)	10.7	10.8	11.1	11.0	0.18	0.84
MPV (fl)	9.69	9.36	8.84	8.96	0.15	0.15
PDW (%)	11.5	11.4	9.87	10.8	0.43	0.53
Leukocytes (10^9/L)	96.2	79.1	86.6	80.0	3.47	0.28
Heterophils (10^9/L)	9.57	8.21	9.21	6.14	0.66	0.26
Lymphocytes (10^9/L)	86.6	70.9	77.4	73.7	3.25	0.36
Thrombocytes (10^9/L)	21.4	19.1	18.2	25.6	1.54	0.37

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, CONT: chicks receiving basal feed and raised under normal density condition (10 chicks per m^2), HSD: chicks receiving basal feed and raised under high-density condition (20 chicks per m²), HSD-P: chicks receiving a feed containing 0.5% germinated papaya seed flour and raised under high-density conditions, HSD-PC: chicks receiving a feed containing 0.5% germinated papaya seed flour and 0.2% chitosan, and raised in high-density condition, SEM: standard error of the means

Table 4: Serum biochemical indices of broiler chickens

Items	CONT	HSD	HSD-P	HSD-PC	SEM	P value
Total cholesterol (g/dL)	97.4	84.6	97.2	107	5.04	0.50
Total triglyceride (g/dL)	49.6	60.1	68.8	76.3	6.94	0.58
LDL (g/dL)	41.6	21.5	29.4	33.1	3.24	0.17
HDL (g/dL)	45.9	52.1	54.0	51.4	1.81	0.44
Total protein (g/dL)	2.58	2.47	2.70	2.78	0.09	0.63
Albumin (g/dL)	1.00	0.99	1.02	1.08	0.03	0.72
Globulin (g/dL)	1.59	1.48	1.68	1.69	0.06	0.61
Uric acid (mg/dL)	5.59	6.13	5.76	4.99	0.30	0.62
Creatinine (mg/dL)	0.03^{c}	0.06^{a}	0.05^{ab}	0.04^{bc}	<0.01	<0.01
AST (U/L)	358	242	251	345	35.3	0.55
ALT (U/L)	2.99	2.08	2.46	2.25	0.21	0.45

^{a,b,c}Means within the same row having different superscripts vary significantly (P<0.05)
LDL: low-density lipoprotein, HDL: high-density lipoprotein, A/G ratio: albumin to globulin ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CONT: chicks receiving basal feed and raised under normal density condition (10 chicks per m²), HSD: chicks receiving basal feed and raised under high-density condition (20 chicks per m²), HSD-P: chicks receiving a feed containing 0.5% germinated papaya seed flour and raised under high-density conditions, HSD-PC: chicks receiving a feed containing 0.5% germinated papaya seed flour and 0.2% chitosan, and raised in high-density condition, SEM: standard error of the means

increasingly limited. This directly reduced feed intake and growth in broiler chickens. In respect particularly to feed intake during days 22-28, chicks at HSD-P and HSD-PC showed greater (P < 0.05) feed consumption as compared to that of HSD chicks resulting in higher (P < 0.05) body weight. The anti-stress property of germinated papaya seed (Ying et al., 2021) and chitosan (Koochacksaraei et al., 2020) seemed to be responsible for the higher feed consumption of the high-stocked broilers. In this case, the antioxidant activities of germinated papaya seed and chitosan were most likely to alleviate stress in chickens, allowing the physiological conditions in the chicken body to be properly maintained. Indeed, Sudarman et al. (2011) documented that less stressed-broiler showed better nutrient digestibility and hence greater feed intake.

Throughout the study period (days 15-28) the feed consumption was highest (P < 0.05) in chicks at normal density pens. The less stress and competition for accessing the feeder accounted for the high feed intake (Sugiharto, 2022). Despite being lower (P < 0.05) than CONT, HSD-P and HSD-PC chickens consumed more (P < 0.05) feed than HSD chickens. In this case, HSD-P and HSD-PC chickens may be less stressed than HSD chickens, resulting in higher feed intake and body weight gain. Although there were no differences (P > 0.05) in final body weight, weight gain, feed intake, or FCR between the two treatments during the study, a mixture of germinated papaya seed and chitosan resulted in a higher (P = 0.06) European Production Higher Efficiency Factor (EPEF) than germinated papaya seed. This result may confirm the synergistic effect between germinated papaya seed and chitosan in improving broilers' physiological conditions and growth under high-density-induced stress. In line with our finding, Al-Baqami and Hamza (2020) previously showed the synergistic effect between chitosan and vanillin in alleviating oxidative stress in male Wistar rats, which was superior to the effect of chitosan or vanillin alone. Apart from the synergistic effect of germinated papaya seed and chitosan, chitosan may be able to act individually in improving the antioxidant and physiological status of chickens, thereby improving broiler chicken growth performance when chickens were stressed due to high stocking density (Chang et al., 2020; Koochacksaraei et al., 2020). With regard to the thinning strategy during the rearing period and the compromised growth performance of broilers stocked at high density during days 22-28, it may be suggested that thinning should be done when the chicken is 21 days old to avoid losses due to decreased growth performance.

This study found that rearing broiler chickens at different densities from day 15 to 28 had no substantial effect (P > 0.05) on broiler complete blood counts (Table 3). In line with our current findings, Sugiharto et al. (2022) have recently documented that raising 18 broiler chickens per square meter had no effect (P > 0.05) on blood profile when compared to raising 9 chicks per square meter. Abouelenien et al. (2016), on the other hand, demonstrated a significant effect of different pen densities on the blood profile of broilers. The latter authors revealed that compared to chicks stocked at 10 chicks per square meter, chicks stocked at 20 chicks per square meter had lower levels of haemoglobin, erythrocytes, total leukocytes, and lymphocytes while increasing levels of heterophils. The data discrepancy between this study and previous studies could be attributed to differences in the body weight of the chickens in pen. Abouelenien et al. (2016) noticed that on day 42, the average body weight of chickens reared at high density was 2,214 g/chick (total weight of chickens per square meter: 44,280 g). Whereas, Sugiharto et al. (2022) revealed that the body weight of chickens ranged from 1,727 to 1,881 g/chick (total weight of chickens per square meter: 31,086-33,858 g). In our study, the body weight of chickens ranged from 1,147-1,243 g/chick (total weight of chickens per square meter: 22,940-24,860 g/chick). Indeed, the total body weight of chickens per square meter in our study was very likely not to exert severe stress so that the physiological homeostasis of chickens has not been significantly disturbed. According to Sugiharto (2022), physiological stress will arise in broiler chickens if the chicken has a total body weight exceeding 33 kg per square meter. Chickens weighing more than 33 kg per square meter can obstruct the movement or access of chickens to the feeder. This can directly result in chickens competing for feed and causing stress. Furthermore, the higher the chicken's body weight in pen, the less smooth the air circulation, the higher the production of ammonia in the cage, and the wetter the litter conditions. This environmental stress can eventually cause stress and disrupt physiological homeostasis in chickens, which may be seen from the blood profile.

While there was no significant effect of the treatments on serum biochemical parameters, the treatments did affect ($P < 0.05$) the serum creatinine concentration of broiler chickens (Table 4). However, it should be noted that the serum creatinine levels in this study were below the normal range, as the typical serum creatinine concentration of broilers range between 0.1 and 0.4 mg/dL Merck (2012). Despite serum creatinine levels that were below the normal range, when compared to controls, chicks raised in high-density pens had higher ($P < 0.05$) serum creatinine concentrations. In most cases, stress has been linked to elevated creatinine levels in broiler chicken blood (Sugiharto, 2020). Huang et al. (2018), for example, found elevated creatinine levels in the blood of heat-stressed broilers. In this case, stress caused by high stocking density appeared to be linked to damage to some internal organs, particularly the kidney (Sugiharto, 2020). According to the latter author, elevated corticosterone levels during stress conditions are usually associated with increased muscle protein catabolism and, thus elevated uric acid and creatinine levels in broiler circulation. While there was no significant reduction in serum creatinine levels in high-stocked broilers supplemented with papaya seed flour, dietary supplementation with a combination of 0.5% germinated papaya seed flour and 0.2% chitosan could lower creatinine levels in broiler serum. Given the lack of difference in serum creatinine between HSD and HSD-P, our findings suggested that chitosan effectively reduces creatinine levels in broilers during stress conditions caused by high stocking density. Ayman et al. (2022) reported a decrease in creatinine in broilers with dietary chitosan supplementation, which is consistent with our findings. Chitosan most likely acted as a stress-relieving agent in high-stocked broilers (Chang et al., 2020), protecting broiler chickens from oxidative tissue damage, particularly in the kidney.

CONCLUSIONS

The use of germinated papaya seeds or a mixture of germinated papaya seeds and chitosan had a positive impact on production performance. It also reduced the negative impact of stress due to high stocking density on kidney health and muscle protein catabolism (based on blood creatinine levels) during rearing from days 15 to 28.

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CONFLICT OF INTEREST

The authors had no conflict of interest.

NOVELTY STATEMENT

This is the first study reporting the benefits of germinated papaya seeds or a mixture of germinated papaya seeds and chitosan in improving broiler performance and kidney health while alleviating muscle protein catabolism in high-density pens from days 15 to 28.

AUTHOR'S CONTRIBUTION

IA, EW, HIW, TY, TAS conducted the experiment and laboratory analysis, SS designed the experiment, drafted and revised manuscript.

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