

# Effectivity of Dry and Liquid BS4 Enzymes in Improving Performance of Broiler Chickens Fed Different Nutrient Density Diet

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## ABSTRAK

Sinurat AP, Haryati T, Pratiwi N, Purwadaria T. 2022. Efektifitas enzim BS4 kering dan basah dalam peningkatan proforma ayam broiler yang diberi pakan kepadatan pakan yang berbeda. *JITV* 27(2):84-92. DOI: <http://dx.doi.org/10.14334/jitv.v27i2.3051>.

Suplementasi enzim ke dalam pakan sudah banyak dilakukan untuk meningkatkan pencernaan gizi pakan dan performa unggas. Suatu enzim (BS4) untuk imbuhan pakan sudah dihasilkan dengan membiakkan *Eupenicilium javanicum*. Efektivitas enzim ini perlu diuji karena banyak faktor yang mempengaruhi efektivitas penambahan enzim ke dalam pakan. Suatu penelitian dilakukan untuk mengetahui efektivitas penambahan enzim BS4 ke dalam pakan terhadap performa ayam broiler. Sebanyak 300 ekor ayam broiler umur sehari dibagi ke dalam 30 kandang dan dipelihara hingga umur 35 hari. Ayam diberi makan ransum percobaan yang merupakan faktorial dari 2 (ransum standard dan ransum kepadatan gizi rendah) X 3 (tanpa enzim, ditambah enzim BS4 cair, ditambah BS4 tepung) dengan 5 ulangan. Pengamatan dilakukan terhadap performa (konsumsi pakan, bobot badan, FCR dan daya hidup) pada periode starter (1-21 h) dan periode selama penelitian (1-35 h). Pada akhir penelitian dilakukan pengukuran persentase karkas, bobot lemak abdomen, rempela dan hati. Hasil menunjukkan bahwa performa ayam 1-35 h tidak dipengaruhi oleh interaksi antara kepadatan gizi dengan penambahan enzim. Kepadatan gizi juga tidak nyata mempengaruhi performa ayam broiler. Penambahan enzim nyata menurunkan konsumsi pakan dan memperbaiki FCR dibandingkan dengan tanpa penambahan enzim. Penambahan enzim BS4 bentuk cair maupun padat menyebabkan penurunan 3,6% konsumsi pakan. Enzim BS4 cair memperbaiki FCR 6,4%, sedangkan BS4 padat memperbaiki FCR 8,9%, namun tidak ada perbedaan yang nyata antara enzim cair dan padat. Perlakuan kepadatan gizi, penambahan enzim maupun interaksi kedua faktor tersebut tidak nyata mempengaruhi persentase karkas, bobot relatif lemak abdomen, hati dan rempela ayam broiler.

**Kata Kunci:** Performa Ayam Broiler, Enzim BS4, Cair, Kepadatan Nutrisi, Padat

## ABSTRACT

Sinurat AP, Haryati T, Pratiwi N, Purwadaria T. 2022. The effectivity of dry and liquid BS4 enzymes in improving performance of broiler chickens fed different nutrient density diet. *JITV* 27(2):84-92. DOI: <http://dx.doi.org/10.14334/jitv.v27i2.3051>.

Supplementation of enzymes in feed is now commonly practiced to increase the nutrient availability of feed and the performance of poultry. A new enzyme called BS4 was produced by cultivating *Eupenicilium javanicum*. It is necessary to test the efficacy of this enzyme since the effectiveness of enzyme supplementation depends on many factors. An experiment was conducted to study the effect of dietary BS4 enzyme supplementation in improving the performance of broiler chickens. A number of 300 broilers DOC was distributed into 30 pens and reared until 35 d. Six experimental diets i.e., factorial of 2 (Standard diet, and low nutrient density diet) X 3 (Control, BS4 liquid enzyme, and BS4 powder enzyme) were formulated with 5 replications. The performance (feed intake, body weight, FCR, and survival rates) were observed during the starter (1-21 d) and whole (1-35 d) periods. At the end of the trial, measurements were also made on the carcass yield, abdominal fat, liver, and gizzard weights. Results showed that performances of broilers from 1-35 d were not significantly affected by interaction between nutrient density and enzyme supplement. The nutrient density also did not affect performances of broilers. However, dietary enzyme supplementation significantly reduced feed intake and improved FCR of broilers as compared to the control. Supplementation of BS4 in liquid or powder form, reduced feed intake by 3.6%. Supplementation of liquid and powder BS4 enzymes improved FCR by 6.4% and 8.9%, respectively, but no different effect between liquid and powder BS4 enzymes on performance of broilers. Nutrient density, enzyme supplementation, and interactions between the two factors did not significantly influence carcass yield, abdominal fat, liver, and gizzard relative weights of broilers.

**Key Words:** Broiler Performances, BS4 Enzyme, Liquid, Nutrient Density, Powder

## INTRODUCTION

Enzymes are composed of proteins that act as biological catalysts to accelerate biochemical reactions. Enzymes are now commonly used in poultry feed as a

supplement to enhance nutrient digestibility of feed, especially those containing feed ingredients with high anti-nutritive factors such as non-starch polysaccharides (NSP). Many enzymes are used in poultry feed production nowadays, either as single- or multi-

enzymes. Supplementing enzymes into feed is expected to improve performance, reduce feed cost and minimize hazardous effect on the environment due to poultry production (Costa et al. 2008; Alagawany et al. 2018). However, not all enzymes supplement showed a similar degree of improvement on chicken performance. There are many factors affecting enzymes supplementation on chickens performance such as the match between enzymes and the presence of antinutrient factors in feed or type of diet, types of enzymes (Saleh et al. 2018), age of the bird, and feed processing (Amerah et al. 2011; Mbukwane et al. 2022). Therefore, it is imperative to study the effectiveness of enzymes in every environmental condition.

The use of enzymes in feed formulation could be applied in two methods. The first method is called “on top” application, i.e., supplementing into the feed without considering the improvement of the available nutrient due to enzyme (Allouche et al. 2015). In this method, the feed was formulated according to the standard nutrient requirement of the birds and added the enzyme as recommended by the producer on top of the formula. Feed price will be more expensive in this method, but it is expected to be compensated by the improvement in the performance of the birds, especially in improving feed conversion ratio due to enzymes supplementation. The second method is by supplementing the enzyme with a diet formulated to contain lower nutrient contents than the standard as shown by some researchers (Yegani & Korver 2013; Saleh et al. 2018). The reduction is expected to be compensated by the enzyme supplemented. With this method, the feed price will be cheaper than the standard formulae and expecting to achieve similar performance to the standard formula. The second method was used in this experiment to study the effectivity of BS4 enzymes on the performance of broiler chickens.

A new multi-enzyme called BS4 has been produced by cultivating *Eupenicillium javanicum* in a solid substrate fermentation. The BS4 contains  $\beta$ -mannanase, cellulase,  $\beta$ -mannosidase, and  $\alpha$ -galactosidase (Sinurat et al. 2014). Studies on the effectivity of the BS4 enzyme on performance of laying hens, growing ducks, and native chickens have been carried out. Reports showed that supplementation of BS4 enzyme in the diet significantly improved the FCR of growing ducks (Purba & Sinurat 2018), and FCR of growing local chickens (Sinurat et al. 2017). All these studies used the BS4 enzyme in liquid form.

Most commercial enzymes are provided in dry powder form, although some are in liquid form. Liquid enzymes have a high-water content which allows the enzymes active all the time and may interact between enzymes that reducing the activity in the case of multi enzymes, especially when the protease is present. High water contents provide more chance of microbial

growth when stored at ambient temperatures. Powdered enzymes are produced by removing the water content or immobilization process which automatically inhibits the enzyme to be active. It is easier to handle for storage, and more resistant to various environmental changes and transportation conditions as compared to liquid enzymes (Homaei et al. 2013). Drying is needed to produce the powder which might reduce its activities during the process and may affect its effectiveness when used as a feed supplement. Therefore, a study was designed to evaluate effectivity of BS4 enzyme when given in liquid or powder form.

## MATERIALS AND METHODS

This experiment was carried out at the Indonesian Research Institute for Animal Production - Ciawi Bogor – Indonesia. All procedures regarding the use of live animals were carried out according to approval by The Animal Welfare Committee at the Indonesian Agency for Agricultural Research and Development. The approval number for this experiment is Balitbangtan/Balitnak /A/02/2016.

The liquid enzymes were produced by cultivating *Eupenicillium javanicum* by solid-state fermentation process as described by Haryati et al. (2019). The enzyme was immobilized by the absorption method to produce powder enzymes (Haryati et al. 2010). In brief, concentrated liquid BS4 enzymes were added to wheat pollard powder (mesh 60) with a 1:1 (v/w) ratio and mixed thoroughly. The mixture was then dried in an oven at 37°C for 2 x 24 hours. Activity of both liquid and powder enzymes was determined by saccharification method as described by Haryati et al. (2019). One unit of saccharification activity is defined as the amount of enzyme in ml or g DM that liberates 1  $\mu$ mol of glucose from palm kernel cake per minute under assay conditions. The amount of enzyme supplemented into the feed have similar activities, i.e., 30 saccharification Unit/kg feed or equal to 1500 ml BS4 liquid enzymes/ton feed, or 2000 g BS4 powder enzymes/ton feed.

Three hundred unsexed broiler DOC were purchased from a commercial hatchery. The chicks were reared on pens covered with rice hulls as litter. Each pen-sized 284 x 146 cm (l x w) was filled with ten chicks, and considered as one experimental unit or replication. Every five pens, were fed with one of the six dietary treatments from 1 to 35 days old.

Two diets, i.e., a standard diet and a low-nutrient density diet were formulated. Standard diet was formulated to fulfill nutrient requirement of broiler chickens, and low nutrient density diet contained a lower protein and ME with a similar ME: protein ratios, i.e., 12.9 and 14.3 kcal ME/g crude protein for starter and grower diet, respectively. The standard starter contained 22.5% crude protein and 2900 kcal ME/kg,

**Table 1.** Ingredients and nutrient composition of starter and grower diets of broiler chicken

Ingredients, %	Starter diet (1-21 day)		Grower diet (22-35 day)	
	Standard	Low Density	Standard	Low Density
Maize	52.64	53.83	59.79	56.83
Meat and bone meal	6.00	4.00	6.00	6.00
Soy bean meal	29.65	30.00	26.90	24.08
Rice bran	7.81	8.67	2.55	10.00
Crude palm oil	2.00	1.00	3.00	1.31
Limestone	0.16	0.20	0.15	0.19
DL-Methionine	0.42	0.40	0.38	0.37
L-Threonine	0.09	0.08	0.05	0.06
L-Lysine	0.36	0.34	0.24	0.27
Dicalcium phosphate	0.30	0.90	0.36	0.31
Premixes (vitamins, minerals and additives)	0.58	0.58	0.58	0.58
Total	100	100	100	100
Calculated nutrient composition				
Dry matter, %	88.00	88.00	88.00	88.1
Crude fiber, %	4.00	4.00	3.42	3.42
Metabolisable energy (ME), kcal/kg	2900	2815	3000	2910
Crude protein (CP), %	22.50	21.80	21.00	20.37
ME: CP ratio, kcal/g	12.90	12.90	14.30	14.30
Calcium, %	0.90	0.90	0.90	0.90
Available Phosphorous, %	0.50	0.50	0.50	0.50
Lysine, %	1.450	1.397	1.255	1.229
Methionine + Cystine, %	1.040	1.000	0.967	0.940
Dig. Lysine, %	1.270	1.232	1.100	1.067
Dig. Methionine + Cystine, %	0.940	0.912	0.870	0.844

and the standard grower contained 21.0% crude protein and 3000 kcal ME/kg. Low-nutrient density contained 21.8% crude protein and 2815 kcal ME/kg for starter diet, 20.37% crude protein, and 2910 kcal ME/kg for grower diet. The starter diets were fed from 1 to 21 days old, followed by the grower diets from 22 to 35 days old. Ingredients and nutrient composition of the diets are presented in Table 1. Each diet was supplemented with either no enzyme, BS4 liquid enzyme, or BS4 powder enzyme.

Performances of the chickens (body weight, feed intake, feed conversion ratios, and mortalities) were observed during starter period (1-21 days) and whole period of the trial (1-35 days). At the end of the feeding trial, one male and one female chicken from each pen were taken randomly and slaughtered to measure carcass percentage, abdominal fat, gizzard, and liver weight. The performance data were subject to analyses

of variance in 2x3 factorial design with five replicates, while data on carcass yield, abdominal fat, gizzard, and liver weights were analyzed in 2x2x3 factorial design with five replications. Further analyses were carried out with the Duncan test to determine the difference between treatments when the ANOVA was significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Results

#### *Growth performance*

Performances of broilers during starter and whole period are presented in Table 2 and Table 3. Feed intake was significantly affected by interaction ( $P < 0.01$ ) between dietary nutrient levels and enzyme

supplementation. Broilers fed with low nutrient diet consumed significantly less feed when supplemented with BS4 enzymes. The highest feed intake was found in broilers fed with no enzyme supplementation (1335 g/bird) and significantly different from those diets supplemented with BS4 powder enzymes (1194 g/bird). Feed intake of broilers on diet supplemented with liquid BS4 enzyme (1313 g/bird) was lower than those fed on diet without enzyme and higher than those fed on diet supplemented with powder BS4 enzyme, although the difference was not significant ( $P>0.05$ ). However, enzyme supplementation did not significantly ( $P>0.05$ ) affect feed intake of broilers when fed with the standard diet.

The treatments (nutrient levels, enzyme supplementation, and their interactions) during starter period did not affect body weight at 21 days old significantly ( $P>0.05$ ). The heaviest broiler weight was found on broilers fed with the low nutrient diet without enzyme supplementation (937 g). The lighter weight was found on broilers fed low nutrient diet and supplemented with BS4 powder enzyme.

The treatments also did not affect FCR ( $P>0.05$ ) during starter period. The lowest FCR (1.381) was achieved by broilers fed standard diet supplemented with BS4 liquid enzymes. On the other hand, broilers fed with the low diet supplemented with BS4 liquid enzyme performed the highest FCR value (1.442). Average body weight of broilers fed with low nutrient diet (902 g/bird) was similar to those fed with standard diet (904 g/bird), but FCR was better on broilers fed with standard diet (1.408) than broilers fed with low nutrient diet (1.424). Average body weight of broilers fed the non-supplemented diet (922 g/bird) was slightly heavier than broilers fed with the liquid supplemented diet (893 g/bird), and BS4 powder enzymes (894 g/bird), although the difference between treatments was not significant. There was no different effect between liquid and powder BS4 enzyme supplementations on body weight at 21 days old. However, average FCR of broilers fed the diet without enzyme was inferior (1.424) to those fed the diet supplemented with BS4 enzyme in liquid (1.412) or powder forms (1.403).

Survival rates of broilers during starter period were also not significantly ( $P>0.05$ ) affected by nutrient levels, enzyme supplementation, and their interactions. Average survival rate of broilers fed with low nutrient levels (97%) was slightly lower than broilers fed with the standard diet (99%). Survival rates were similar on birds fed without enzyme supplement (98%) with broilers fed enzyme supplement in liquid (98%) or powder forms (99%). Nonetheless, the survival rates in all treatments were still high.

Effect of treatments on performances of broilers during the whole period (1 to 35 days old) is presented in Table 3. Interaction between nutrient level and enzyme supplement did not significantly ( $P>0.05$ )

influence feed intake, body weight, FCR, and survival rates of broilers during the period. Therefore, performance data presented to describe the main effect only.

Feed intake of broilers from 1 to 35 days was not significantly ( $P>0.05$ ) affected by the nutrient density. Average feed intake of broilers fed the low diet was slightly higher (3214 g/bird) than the standard (3163 g/bird). However, feed intake of broilers from 1 to 35 d was significantly ( $P<0.05$ ) affected by dietary enzyme supplementation. Broilers fed the diet without BS4 enzymes consumed (3275 g/bird) more feed than broilers fed the diet with BS4 enzymes. There was no significant difference in feed intake of broilers fed the diet with the liquid (3156 g/bird) and the powder (3135 g/bird) BS4 enzymes.

Broilers' body weight at 35 d of age was not significantly ( $P>0.05$ ) affected by nutrient density, enzyme supplement nor by interactions between the two factors. Average body weight of broilers at 35 days fed with low nutrient density was slightly heavier (1966 g/bird) than those fed the standard diet (1940 g/bird). Average body weight of broilers fed the diet without enzyme supplement was slightly lower (1909 g/bird) than those fed diet supplemented with liquid (1958 g/bird) or powder (1992 g/bird) BS4 enzyme.

FCR of broilers from 1 to 35 d was not significantly ( $P>0.05$ ) influenced by the nutrient density. The average FCR of broilers fed the low diet was similar (1.639) to those fed standard diet (1.636). However, enzyme supplementation significantly ( $P<0.05$ ) influenced the FCR. Average FCR of broilers from 1 to 35 days was higher (1.719) or less efficient than broilers fed the diet supplemented with enzymes. FCR of broilers fed the diet supplemented with BS4 liquid enzyme (1.615) was slightly higher than those supplemented with BS4 powder enzyme (1.579) although the difference was not significant ( $P>0.05$ ).

Survival rates of the broilers from 1 to 35 d were not significantly ( $P>0.05$ ) influenced by interaction between nutrient density and enzyme supplementation, nutrient density nor by enzyme supplementation. Average survival rate was between 96 – 100% and 93 – 97% during starter, and the whole period, respectively.

### ***Carcass yield and organ weight***

Effect of treatments on carcass percentage, abdominal fat levels, gizzard weight, and liver of broilers at 35 d of age are presented in Table 4. The nutrient density, enzyme supplementation, sex, or interactions between the two factors did not significantly ( $p>0.05$ ) affect carcass percentage, abdominal fat level, gizzard weight, and liver weight of male and female broilers. Only live bodyweight of the broilers was significantly ( $p<0.01$ ) affected by sex.

**Table 2.** Performance of broilers fed with different nutrient density and enzyme supplement during starter period (1-21 day)

Nutrient level	Type of Enzyme	BW DOC g/bird	Feed intake, g/bird	BW 21 d, g/bird	FCR	Survival rate, %
Low Nutrients	None	50.8±2.5	1335±59 <sup>a</sup>	937±91	1.436±45	96.0±5.5
	Liquid	51.9± 0.5	1313±90 <sup>ab</sup>	912±79	1.442±0.065	96.0±5.5
	Powder	50.1±5.2	1194±33 <sup>b</sup>	857±23	1.394±0.042	100.0±0.0
Standard Nutrients	None	50.1±2.5	1298±58 <sup>ab</sup>	907±30	1.432±0.084	100.0±0.0
	Liquid	48.9±4.2	1206±81 <sup>ab</sup>	874±56	1.381±0.063	100.0±0.0
	Powder	48.9±5.7	1312±74 <sup>a</sup>	931±45	1.411±0.087	98.0±4.5
Significance (P):						
Nutrient Level (N)		0.27	0.73	0.93	0.63	0.15
Type of Enzyme (E)		0.83	0.09	0.47	0.72	0.78
N x E		0.79	<0.01	0.08	0.60	0.13

BW= body weight, DOC= day old chick, FCR= food conversion rate. Means in the same column with different superscript differ significantly (P<0.05)

**Table 3.** Performance of broilers fed with different nutrient density and BS4 enzyme supplement during experimental period (1-35 day)

Treatments	Feed intake 1-35 day, g/bird)	Body weight 35 day, g/bird	FCR	Survival rate, %	
Effect of Nutrient Density:					
Low	3214 ±128	1966 ±107.0	1.639 ±0.097	94.0 ±7.4	
Standard	3163 ±150	1940 ±89.7	1.636 ±0.110	95.3 ±7.4	
Effect of enzymes supplementation:					
None	3275 ±87 <sup>a</sup>	1909 ±85.0	1.719 ±0.117 <sup>a</sup>	97.0 ±6.7	
Liquid	3156 ±169 <sup>b</sup>	1958 ±84.4	1.615 ±0.065 <sup>b</sup>	93.0 ±6.7	
Powder	3135 ±117 <sup>b</sup>	1992 ±110.2	1.579 ±0.096 <sup>b</sup>	94.0 ±8.4	
Significance (P):					
Nutrient Density (N)		0.27	0.49	0.93	0.63
Enzyme (E)		0.04	0.23	0.03	0.48
N x E		0.11	0.44	0.97	0.48

FCR= food conversion rate. Means in the same column and factor with different superscript differ significantly (P<0.05)

Average body weight of males (2297 g/bird) was heavier than female broilers (1987 g/bird). The nutrient density and the enzyme supplementation did not significantly (P>0.05) affect body weight, carcass yield, abdominal fat, gizzard, and liver weight.

## Discussion

Feeding broilers with a lower nutrient density than the standard recommendation will impair the performance of the broilers. Supplementing enzymes into the low nutrient density diet is expected to restore the

performance. However, the result of the present study did not show a significant difference between the standard and the low nutrient density diets. Delezie et al. (2010) reported that broilers fed with a lower nutrient density than the standard diet with a similar ME protein ratio depressed feed intake, body weight gains, and impaired FCR when ME was reduced too drastic (300 kcal/kg). However, when the difference was not too drastic (150 kcal/kg), the performances were not significantly affected by nutrient density.

Abdollahi et al. (2018) also reported that there was only a slight reduction in performance of broilers when

**Table 4.** Carcass and some organs weight of broilers fed with different nutrient levels and enzyme supplementation

Treatments	Live body weight	Carcass. g/100 g BW	Abdominal fat. g/100 g BW	Gizzard. g/100 g BW	Liver g/100 g BW
Nutrient density (N)					
Standard	2107±234	66.7±2.676	1.03±0.276	1.82±0.25	2.32±0.37
Low	2177±243	67.9±2.231	0.98±0.278	1.76±0.28	2.25±0.37
Enzymes (E)					
None	2130±220	67.8±2.414	1.07±0.273	1.75±0.23	2.37±0.42
Liquid	2170±261	66.9±2.241	1.00±0.230	1.86±0.23	2.28±0.41
Powder	2125±245	67.3±2.890	0.95±0.292	1.76±0.32	2.21±0.25
Effect of Sex (S)					
Female	1987±144 <sup>a</sup>	67.2±2.615	1.04±0.256	1.82±0.25	2.31±0.32
Male	2297±214 <sup>b</sup>	67.5±2.448	0.96±0.294	1.76±0.28	2.26±0.41
Significance (P)					
Nutrient density (N)	0.14	0.08	0.45	0.34	0.44
Enzyme (E)	0.69	0.54	0.41	0.33	0.38
Sex (S)	<0.01	0.69	0.28	0.34	0.59
N x E	0.92	0.84	0.92	0.42	0.15
N x S	0.86	0.94	0.780	0.31	0.96
E x S	0.22	0.90	0.42	0.94	0.16
N x E x S	0.11	0.39	0.34	0.06	0.60

BW= body weight. Means in the same column and factor with different superscript differ significantly (P<0.05)

ME of the diet was reduced by 100 kcal/kg, but the performance reduction was significantly higher when the ME of the diet was 200 kcal/kg lower than the standard diet. Alqhtani et al. (2022) showed that the feed intake and bodyweight gain of broilers were not significantly affected by diet density when the difference of the ME was less than 100 kcal/kg. The ME difference between the standard and the low diet in the present experiment was only 100 kcal/kg. These may be the reasons why there was no difference in performance of broilers fed diet with different densities.

This study showed an interaction effect between enzyme supplementation and nutrient density on feed intake during the starter period but not during the whole period, which indicated that the supplementation effectively reduced feed intake when fed low nutrient density diet during starter period. However, Abudabos (2012) and Alqhtani et al. (2022) showed no interaction between nutrient density and enzyme supplementation on feed intake of broilers during the starter period. No explanation could be described at this stage for this discrepancy.

Beneficial effect of enzyme supplementation in broiler feed is normally expressed in improvement in feed efficiency utilization or reduction of feed conversion ratio (FCR). In general, nutrient digestibility

of the diet was low in the early stage of life and increases with increasing age for chicks (Batal & Parsons 2002). This may be related to development of digestive tract and organs related to digestion process, and it is expected that effect of enzyme supplementation on feed efficiency is higher when given in starter period than during grower or whole (starter and grower) period. However, different results were found in the present study and other studies (Abudabos 2012; Alqhtani et al. 2022). The present study showed average improvement in FCR was 6.7%, and 7.1% during starter period (1-21 d), and whole period (1-35 d), respectively. Abudabos (2012) showed 1.3% and 6.0% FCR improvement during starter (1-22 d) and whole period (1-42 d), respectively. Alqhtani et al. (2022) reported 1.6% and 2.8% FCR improvement during starter (1-22 d) and the whole period (1-42 d), respectively. Perhaps, more studies are required to explain this phenomenon.

Supplementation of BS4 enzyme did not influence body weight during the starter and the whole period but significantly reduced feed intake and improved FCR of broilers during whole experimental period, regardless of the nutrient density. Feed intakes were reduced by 119 g/bird (or 3.6% of the standard), and 140 g/bird (or 4.3% of the standard) when BS4 liquid- and powder

enzymes were supplemented, respectively. FCR was 6.4% and 8.9% better than the standard as effect of BS4 liquid or powder supplement, respectively. These result indicated that the BS4 enzyme increased availability of nutrients in the diet, hence less feed was required to perform similar bodyweight as standard. It has been reported that BS4 enzymes mainly consist of carbohydrates such as  $\beta$ -mannanase, cellulase,  $\beta$ -mannosidase, and  $\alpha$ -galactosidase (Sinurat et al. 2014). However, the enzymes are capable to increase metabolizable energy (digestibility of carbohydrates), protein, and amino acids of feed ingredients or feed (Pasaribu et al. 2009; Sinurat et al. 2013; 2014; 2015).

Some variations in the poultry performance improvement due to supplementation of BS4 enzymes in the feed have been reported. Supplementation of BS4 enzymes in commercial laying hen's feed increased by 3.2% egg production and improved by 5.8% FCR. There was no improvement in egg production, but FCR was improved by 4.3% in laying native chickens (Sinurat et al. 2019). Bodyweight gain was increased by 7.2% and the FCR by 6.8% in growing ducks (Purba & Sinurat 2018). Bodyweight gain was not affected, but FCR improved by 2.2% in growing native chickens (Sinurat et al. 2017).

A meta-analysis was conducted based on 21 published reports to study effect of multi-carbohydrases containing  $\alpha$ -galactosidase + xylanase or  $\alpha$ -galactosidase +  $\beta$ -glucanase on improving performance of broilers' (Llamas-Moya et al. 2021). This study concluded that multi-carbohydrases have a significant effect on improvement of broilers performance. Body weight gain increased by 56 g/bird, and FCR reduced by 0.042 or 2.4% better than performance of broilers without enzyme supplements. Result of the present study showed that body weight increased by 49, and 83 g/bird when supplemented with BS4 enzymes in liquid, and powder, respectively. The results also showed an improvement in FCR 0.104 (or 6.4%) and 0.14 (or 8.9%) with BS4 enzymes supplementation in liquid, and powder form, respectively. These results indicated effectivity of BS4 enzymes in liquid or powder form to improve the performance of broilers is better than the average effectivity of multi-carbohydrases reported.

Reports on effect of powder and liquid enzymes are scant. The present study showed a slightly better improvement of powder than the liquid BS4 enzymes in improving broiler's performance, but the difference was not significant. Sinurat et al. (2019) also found similar results when BS4 enzymes were supplemented into the diet of native chicken layers. The present study showed that the effectivity of enzymes did not depend on the form but their enzyme activities. Formation process of powder enzyme was immobilization of liquid BS4 on wheat pollard with adsorption method. In this method, the enzyme is not firmly bound to the matrix. Although immobilization process may affect enzyme activity,

effect on performance of broilers was not shown since liquid and powder BS4 enzymes added were similar, i.e., 30 saccharification Units/kg feed.

There were some studies on effect of nutrient density on carcass yield and internal organs of broilers with inconsistent results. The present study showed similar results as reported by Kamran et al. (2008); Zhai et al. (2013); and Kim et al. (2016), which showed that carcass yield, relative weights of abdominal fat, and liver were not affected by nutrient density. On the other hand, Li et al. (2010) showed that broilers fed with a lower nutrient density produced lower abdominal fat levels.

Effect of dietary enzyme supplementation on carcass and internal organ characteristics of broiler chickens has been reported by many authors. Most of the studies showed no significant effect of enzyme supplementation on carcass and organs characteristic of broilers. Dietary enzyme supplementation, especially the carbohydrases are expected to increase AME of the diet and may imbalance ME: protein intake ratios if digestibility of protein is not increased. Diets with a higher ME: protein ratio than the standard is well known to increase abdominal fat deposition in broilers (Nahashon et al. 2005; Hada et al. 2013; Fouad & El-Senousey, 2014). This phenomenon did not occur in the present study. The ME: protein ratio imbalances might not occur since the BS4 enzyme improved ME, protein, and amino acids digestibility of the feed (Pasaribu et al. 2009; Sinurat et al. 2014). Emadinia et al. (2014) and Mohammadigheisar et al. (2021) also reported similar results.

Enzymes supplementation helps digest feed in the digestive tract of chickens by biochemical reactions. Activity of digestive organs may be less as compared to those fed without enzyme supplementation. Therefore, smaller size of organs related to the digestion process is expected in chickens fed dietary enzymes. Some reports support this hypothesis, but not all. Agboola et al. (2013) showed no significant effect of dietary enzyme supplementation on carcass and liver weight but reduced gizzard size of broilers. Some researchers reported that adding enzymes to diets did not affect relative weight of liver and gizzard of broiler chickens (Hassanein 2011; Emadinia et al. 2014; Mohammadigheisar et al. 2021). The present study also showed that BS4 enzyme supplementation did not significantly influence relative weight of liver and gizzard of broilers.

## CONCLUSION

Based on the results, it is concluded that nutrient density did not affect performances (feed intake, body weight, FCR, and survival rates), carcass yield, abdominal fat-, gizzard-, and liver- relative weights of broilers. Supplementation of BS4 enzymes in broilers'

diet reduced feed intake and improved FCR. Effectivity of liquid and powder BS4 enzymes in improving performance of broiler chickens was similar.

## REFERENCES

- Abdollahi MR, Zaefarian F, Ravindran V, Selle PH. 2018. The interactive influence of dietary nutrient density and feed form on the performance of broiler chickens. *Anim. Feed Sci. Technol.* 239:33–43. DOI:10.1016/j.anifeedsci.2018.03.005.
- Abudabos AM. 2012. Effect of enzyme supplementation to normal and low density broiler diets based on corn-soybean meal. *Asian J. Anim. Vet. Adv.* 7:139–148. DOI:10.3923/ajava.2012.139.148.
- Agboola A, Fagbemi M, Oluwadamilare E, Majolagbe O. 2013. Effect of Roxazyme G2G on broiler fed wheat based diet. *Inter J Agri Biosci.* 2:321–326.
- Alagawany M, Elnesr SS, Farag MR. 2018. The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. *Iran. J. Vet. Res.* 19:157–164.
- Allouche L et al. 2015. Effect of addition of exogenous enzymes in hypocaloric diet in broiler chicken on performance, biochemical parameters and meat characteristics. *Biotechnol. Anim. Husb.* 31:551–565. DOI:10.2298/bah1504551a.
- Alqhtani AH, Sulaiman ARA, Alharthi AS, Abudabos AM. 2022. Effect of Exogenous Enzymes Cocktail on Performance, Carcass Traits, Biochemical Metabolites, Intestinal Morphology, and Nutrient Digestibility of Broilers Fed Normal and Low-Energy Corn–Soybean Diets. *Animals.* 12. DOI:10.3390/ani12091094.
- Amerah AM, Gilbert C, Simmins PH, Ravindran V. 2011. Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *Worlds. Poult. Sci. J.* 67:29–46. DOI:10.1017/S0043933911000031.
- Batal AB, Parsons CM. 2002. Effects of age on nutrient digestibility in chicks fed different diets. *Poult. Sci.* 81:400–407. DOI:10.1093/ps/81.3.400.
- Costa FGP, Goulart CC, Figueiredo DF, Oliveira CFS, Silva JHV. 2008. Economic and environmental impact of using exogenous enzymes on poultry feeding. *Int. J. Poult. Sci.* 7:311–314. DOI:10.3923/ijps.2008.311.314.
- Delezie E, Bruggeman V, Swennen Q, Decuyper E, Huyghebaert G. 2010. The impact of nutrient density in terms of energy and/or protein on live performance, metabolism and carcass composition of female and male broiler chickens of two commercial broiler strains. *J. Anim. Physiol. Anim. Nutr. (Berl).* 94:509–518. DOI:10.1111/j.1439-0396.2009.00936.x.
- Emadinia A et al. 2014. Effect of wet feeding and enzyme supplementation on performance and immune responses of broiler chicks. *J. Appl. Anim. Res.* 42:32–37. DOI 0.1080/09712119.2013.795901.
- Fouad AM, El-Senousey HK. 2014. Nutritional factors affecting abdominal fat deposition in poultry: A review. *Asian-Australasian J. Anim. Sci.* 27:1057–1068. DOI: 10.5713/ajas.2013.13702.
- Hada FH et al. 2013. Effect of protein, carbohydrate, lipid, and selenium levels on the performance, carcass yield, and blood changes in broilers. *Rev. Bras. Cienc. Avic.* 15:385–394. DOI:10.1590/S1516-635X2013000400014.
- Haryati T, Marbun P, Purwadaria T. 2010. Preservasi xilanase *Bacillus pumilus* PU4-2 dengan teknik imobilisasi pada pollard dan penambahan kation. *JITV.* 15:63–71.
- Haryati T, Sinurat AP, Hamid H, Purwadaria T. 2019. Optimization of BS4 Enzyme production with Different Substrate Thickness and Type of Trays. *J. Ilmu Ternak dan Vet.* 24:158. DOI:10.14334/jitv.v24i4.2055.
- Hassanein HH. 2011. Growth Performance and Carcass Yield of Broilers as Affected by Stocking Density and Enzymatic Growth Promoters. *Asian J. Poult. science.* 5:94–101.
- Homaei AA, Sariri R, Vianello F, Stevanato R. 2013. Enzyme immobilization: An update. *J. Chem. Biol.* 6:185–205. DOI:10.1007/s12154-013-0102-9.
- Kamran Z et al. 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. *Poult. Sci.* 87:468–474. DOI:10.3382/ps.2007-00180.
- Kim SJ, Lee KW, Kang CW, An BK. 2016. Growth performance, relative meat and organ weights, cecal microflora, and blood characteristics in broiler chickens fed diets containing different nutrient density with or without essential oils. *Asian-Australasian J. Anim. Sci.* 29:549–554. DOI:10.5713/ajas.15.0426.
- Li W Bin et al. 2010. Influence of lighting schedule and nutrient density in broiler chickens: Effect on growth performance, carcass traits and meat quality. *Asian-Australasian J. Anim. Sci.* 23:1510–1518. DOI:10.5713/ajas.2010.10087.
- Llamas-Moya S, Higgins NF, Adhikari R, Lawlor PG, Lacey S. 2021. Effect of multicarbohydrase enzymes containing  $\alpha$ -galactosidase on the growth and apparent metabolizable energy digestibility of broiler chickens: a meta-analysis. *Anim. Feed Sci. Technol.* 277:114949. DOI:10.1016/j.anifeedsci.2021.114949.
- Mbukwane MJ, Nkukwana TT, Plumstead PW, Snyman N. 2022. Sunflower meal inclusion rate and the effect of exogenous enzymes on growth performance of broiler chickens. *Animals.* 12:253. DOI:10.3390/ani12030253.
- Mohammadigheisar M, Shouldice VL, Balasubramanian B, Kim IH. 2021. Effect of dietary supplementation of  $\beta$ -mannanase on growth performance, carcass characteristics, excreta microflora, blood constituents, and nutrient ileal digestibility in broiler chickens. *Anim. Biosci.* 34:1342–1349. DOI:10.5713/ab.20.0355.
- Nahashon SN, Adefope N, Amenyenu A, Wright D. 2005. Effects of dietary metabolizable energy and crude protein concentrations on growth performance and carcass characteristics of French guinea broilers. *Poult. Sci.* 84:337–344. DOI:10.1093/ps/84.2.337.



- Pasaribu T, Sinurat AP, Purwadaria T, Ketaren P. 2009. Peningkatan nilai gizi solid heavy phase sebagai pengganti jagung dalam pakan unggas. *JITV*. 14:167–176.
- Purba M, Sinurat AP. 2018. Effect of supplementation of BS4-enzyme levels in rice-bran based rations on performance of growing PMp broiler duck. *JITV*. 23:38. DOI:10.14334/jitv.v23i1.1669.
- Saleh AA, El-Far AH, Abdel-Latif MA, Emam MA, Ghanem R, El-Hamid HS. 2018. Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PLoS One*. 13. DOI:10.1371/journal.pone.0198085.
- Sinurat A, Hidayat C, Haryati T, Wardhani T, Sartika T. 2017. Pemberian enzim BS4 untuk meningkatkan performa ayam KUB masa pertumbuhan. 400–406. DOI:10.14334/pros.semnas.tpv-2017-p.402-408.
- Sinurat AP, Haryati T, Pasaribu T, Sartika T. 2019. Efektivitas enzim BS4 sebagai pengganti antibiotik imbuhan pakan untuk meningkatkan efisiensi pakan pada ayam KUB petelur. 596–602. DOI:10.14334/pros.semnas.tpv-2019-p.596-602.
- Sinurat AP, Purwadaria T, Ketaren PP, Pasaribu T. 2014. Substitutions of soybean meal with enriched palm kernel meal in laying hens diet. *JITV*. 19:184–192. DOI:10.14334/jitv.v19i3.1081.
- Yegani M, Korver DR. 2013. Effects of corn source and exogenous enzymes on growth performance and nutrient digestibility in broiler chickens. *Poult. Sci*. 92:1208–1220. DOI:10.3382/ps.2012-02390.
- Zhai W, Peebles ED, Mejia L, Zumwalt CD, Corzo A. 2013. Effects of dietary amino acid density and metabolizable energy level on the growth and meat yield of summer-Reared broilers. *J. Appl. Poult. Res*. 23:501–515. DOI:10.3382/japr.2014-00961.