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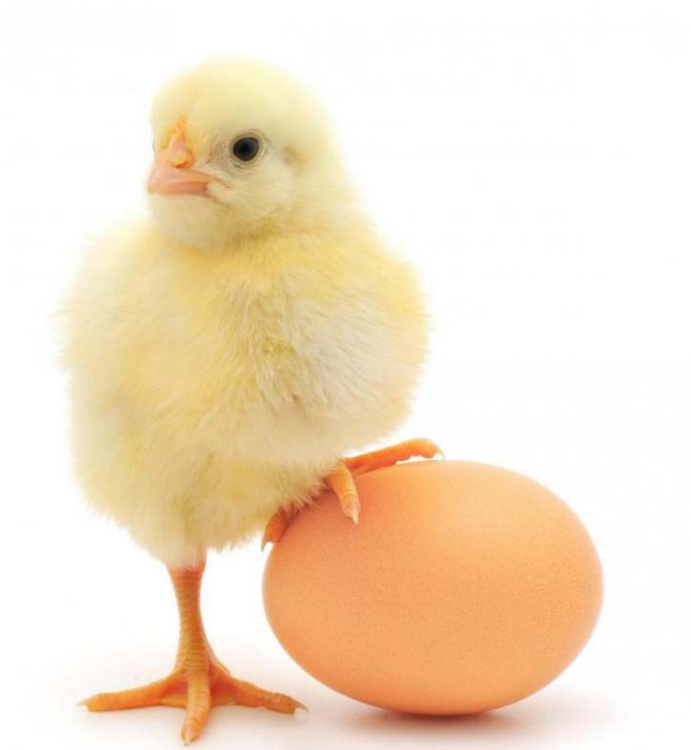

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Research Article
Growth Performance, Feed Digestibility and Meat Selenium of Broilers Fed Fungi-Fermented Rice Bran with Addition of Inorganic Selenium

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Abstract
Objective: A study was conducted to determine effects of fermented rice bran by *Saccharomyces cerevisiae* and *Aspergillus niger* with addition of selenium on growth performance, feed digestibility and meat selenium of broilers. **Materials and Methods:** A finely ground rice bran with addition of 0.1% sodium selenite was autoclaved at 20 psi for 20 min and added distilled water to meet 80% moisture content. The autoclaved substrate was fermented with *Aspergillus niger* and *Saccharomyces cerevisiae* for 5 days to produce *Aspergillus niger*-fermented rice bran (ANFRB) and *Saccharomyces cerevisiae*-fermented rice bran (SCFRB) respectively. A total of 140 day-old-chicks were used and kept for 6 weeks. The birds were fed 5 experimental diets *ad libitum*. The experimental diets were basal diet (T1), Basal diet +0.4 ppm selenium from sodium selenite (T2), basal diet +0.4 ppm selenium from commercial organic selenium (T3), basal diet +0.4 ppm selenium from SCFRB (T4) and basal diet +0.4 selenium from ANFRB (T5). The study used a completely randomized design with 5 treatments and 4 replications. **Results:** Birds fed the T2 diet had the lower body weight gain, feed intake, feed conversion ratio, digestible dry matter intake, selenium intake and selenium digestibility. The birds fed the T3, T4 and T5 diets had higher concentration of meat selenium than those birds fed the T1 and T2 diets. **Conclusion:** Addition of fungi-fermented rice bran with additional sodium selenite in the diets improved the feeding values of diets as efficiently as commercial organic selenium-supplemented diet.

Key words: Fermentation, fungi, poultry, rice bran, selenium

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Abundant production of agricultural by products in countries where their people rely much on agricultural sector along with increased price of conventional feedstuffs commonly used in poultry feed has driven animal nutritionists to selectively use agricultural by-products. It is a fact that the agricultural sector was a rural-based business sector. Replacing conventional feedstuffs with low quality agricultural by-products in poultry diets could downgrade feeding values of the diets due to the presence of anti-nutrients. Studies on the use of agricultural by products in poultry feed have come up with limited success. The problems of additional agricultural by-products were found in rice bran[1], palm kernel meal[2], feather meal[3], copra meal[4] and shallot by products[5]. The presence of anti-nutrients, high dietary fibre, low bulk density, high water holding capacity and slower passage rate in the digestive tract are the chemical and physical factors negatively affecting feeding values of the diets[6]. Accordingly, coping with the problems to improve feeding value of the by-products could financially empower rural community as agriculture sector is a rural based business.

The use of urea as a fertilizer or for non-protein nitrogen source for ruminant animals indicated that microbes have the capacity to convert inorganic nitrogen (non protein nitrogen) into organic protein (microbial protein). Since inorganic minerals could not be utilized by monogastric animals, even are harmful, it is an urgent to find a way to produce organic minerals from inorganic compounds. The same logic can be used to produce organic selenium from inorganic minerals, such as sodium selenite through the use of microorganisms. This has been an important issue as organic selenium is present in very low quantity in many feedstuffs. Poultry fed selenium-deficient diets could suffer from muscular dystrophy, poor body weight, myopathies of gizzard and heart and mortality[7,8].

Efforts have been made to find a technology compatible to feed and poultry industries. Among technologies available, an old technology of solid state fermentation has received more interest from animal nutritionists due to its practicality, low cost and superior products[9]. The use of agricultural by products as solid substrates makes this technology even more attractive, especially in Indonesia where people are dependant their income upon agricultural sector. The efficacy of this technology to increase feeding value of the agricultural by-products-supplemented diets was found in the use of coconut by products[10], palm kernel meal and rice bran[11]. The improved feeding value of fermented agricultural by-products is through bioconversion of undigested substances to easily digested compounds, from inorganic minerals to organic fractions, improved aroma and flavour of substrates and toxins elimination[11]. The fermentation technology could not only improve poultry production but also increase income of the agricultural by products-producing farmers, living mostly in the rural areas. Accordingly, a study was conducted to determine the growth performance of broilers fed fungi-fermented rice bran with or without inorganic selenium addition.

MATERIALS AND METHODS

Fermentation of rice bran: Two different fungi (*Aspergillus niger* and *Saccharomyces cerevisiae*) were used in this study. These fungi were purchased from the Microbiology Laboratory, Science Faculty, Tadulako University. Rice bran was collected from the local market and used as a solid substrate. Prior to fermentation, the rice bran was ground finely to 1-2 mm size. A procedure of solid state fermentation by Gandjar[12] was used in this study. The fine ground rice bran was autoclaved at 20 psi for 20 min. The autoclaved rice bran was cooled to room temperature. The rice bran was either mixed with or without 0.1% sodium selenite. The mixture was added with distilled water to increase the moisture to 80% prior to incubation. The substrates were put in 2-kg plastic bags and incubated with either 0.1% *Aspergillus niger* or 0.1% *Saccharomyces cerevisiae* for 5 days with temperature of 35°C and 85% humidity. The incubated substrates were collected and oven-dried for 48 hours at 50°C. The dried fermented rice bran was stored for proximate analysis[13].

Animals and diets: A total of 140 day old broiler chicks were used in this study. The broiler chicks were initially placed in 5 brooder pens for a week. The birds were then transferred into 20 pens and kept them for another 5 weeks. During brooding, all the broiler chicks were vaccinated against New Castle Diseases at day 3. The broilers were offered basal starter and grower diets. The basal diet as seen in Table 1 was formulated by using a UFFF software package[14]. The experimental diets (Table 2) and drinking water were fed *ad libitum*. A plastic drinker and a plastic feeder were placed inside the pen. The feed in the feeder was topped up twice a day at 07.30 am and 4.30 pm. The drinker was cleaned whenever necessary. The pens and surroundings were kept clean throughout the study.

Parameters measured: The parameters observed were: final body weight, feed intake, feed conversion ratio, selenium intake, dry matter feces, dry matter digestibility, digestible dry

Table 1: Basal diet

	Quantity(%)	
Feed ingredients	Starter	Grower
Soybean meal	19.5	19.30
Maize	61.6	65.50
Fish meal	3.0	3.00
Meat bone meal	12.0	9.00
Rice bran	1.0	1.00
Palm oil	2.0	1.50
Dicalcium phosphate	0.1	0.10
Salt	0.2	0.20
Methionine	0.2	0.10
Lysine	0.2	0.10
Premix	0.2	0.20
Calculated nutrients		
Crude protein	22.50	21.20
Metabolizable energy (kcal kg^{-1})	3012.00	3015.00
Methionine	0.56	0.45
Lysine	1.29	1.20
Selenium	0.13	0.13
Calcium	1.51	1.20
Phosphorus	0.79	0.67

Table 2: Experimental diets

Treatments	Replicates	Birds
T-1: control	4	4
T-2: control+0.4 ppm selenium from sodium selenite	7	7
T-3: control+0.4 ppm selenium from sel-plex	4	7
T-4: control+0.4 ppm selenium from *S. cerevisiae*-fermented rice bran	4	7
T-5: control+0.4 ppm selenium from *A. Niger*-fermented rice bran	4	7

Sel-plex (commercial organic selenium), *S. cerevisiae* (*Saccharomyces cerevisiae*), *A. niger* (*Aspergillus niger*)

matter intake, selenium digestibility and meat selenium. On day 35, two broiler chickens per pen were randomly selected. The broilers were transferred into individual metabolic pens for digestibility measurement. The digestibility data were collected for three consecutive days. Plastic trays were placed underneath the metabolic pens for fecal collection. The feces was collected after discarding any contamination. Feathers and other contamination were hand-picked and discarded from feces prior to weighing. The total feces were oven-dried at 50°C for three days to measure dry matter feces. The 3 day samples from each replicate pen were pooled and finely ground. The ground samples were analysed for selenium according to the method of Almeida *et al.*[15]. Dry matter and selenium digestibilities were calculated based on the total feces collection method.

At the end of the experiment, four birds per replicate cage were randomly selected for measurement of meat selenium. The selected birds were killed by cervical dislocation. The killed birds were dressed by removing the skin and feathers, neck and shank. Breast muscle of the dressed broilers were individually weighed and oven dried at 50°C for 3 days. The dried breast muscle of broiler were chopped and finely ground for analysis of selenium.

Experimental design and statistical analysis: The experiment used a Completely Randomized Design with 5 treatment diets and 4 replications[16]. A one-way Analysis of variance was used to determine treatment effect on measured variable by using Mintab 16. Any differences identified in the variance analysis was further tested with Tukey test using the same statistical software with the significance level of 5%.

RESULTS AND DISCUSSION

Effects of fermentation on nutrient profiles: Data of nutrient contents of rice bran fermented, either by *Aspergillus niger* or *Saccharomyces cerevisiae* are shown in Table 3. Body weight gain, feed intake and feed conversion ratio of birds fed the experimental diets were shown in Table 4. Data of dry matter feces, dry matter digestibility and digestible dry matter intake can be seen in Table 5. Selenium intake, selenium digestibility and meat selenium of broiler chickens are shown in Table 6.

Protein content of indonesian rice bran was 12.1% with crude fibre of 10.6%[17]. Our current finding on protein content was far below the finding of Supriyati *et al.*[17]. This discrepancy might be due to high contamination of rice hull and thus

Table 3: Nutrients content of coconut dregs or fermented coconut dregs (%)

Nutrients	Rice bran (RB)	RB +0.1% Se, fermented by *S. cerevisiae*	RB +0.1% Se, fermented by *A. niger*
Protein (%)	5.10	10.4	9.4
Crude fibre (%)	14.30	11.6	11.8
Lipid (%)	7.10	8.8	8.6
Calculated selenium (ppm)	0.95	1000.0	1000.0
Analysed selenium (ppm)	0.95	658.0	732.0
Biomass Loss (%)	0.00	9.6	6.4

Table 4: Growth performance of birds fed the experimental diets

	Body weight gain (g)			Feed Intake (g)			Feed conversion ratio		
Treatments	Week 4	Week 5	Week 6	Week 4	Week 5	Week 6	Week 4	Week 5	Week 6
T-1	1133[a]	1561[a]	2028[a]	1961[a]	2798	3669[a]	1.73[a]	1.79[ab]	1.81[b]
T-2	242[b]	349[b]	482[b]	419[b]	630	853[b]	1.74[a]	1.81[a]	1.86[a]
T-3	1107[a]	1603[a]	2100[a]	1732[a]	2741	3705[a]	1.57[b]	1.71[c]	1.76[c]
T-4	1067[a]	1508[a]	2040[a]	1751[a]	2655	3632[a]	1.64[ab]	1.76[abc]	1.78[bc]
T-5	1056[a]	1561[a]	2031[a]	1670[a]	2701	3604[a]	1.58[b]	1.73[bc]	1.77[c]
SEM	38.7	29.2	38.8	62.9	54.5	70.2	0.025	0.014	0.010
p-value	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	0.001	>0.001

T-1: Control diet, T-2: Control diet +0.4 ppm selenium from sodium selenite, T-3: Control diet+0.4 ppm selenium from sel-plex (commercial organic selenium), T-4: Control diet +0.4 ppm selenium in the form of sodium selenite) from *Saccharomyces cerevisiae*-fermented rice bran, T-5: 0.4 ppm selenium in the form of sodium selenite from *Aspergillus niger*-fermented rice bran

Table 5: Dry mater feces, dry matter digestibility and digestible dry matter intake of broilers fed the experimental diets

Treatments	Dry matter feces (%)	Dry matter digestibility (%)	Digestible dry matter intake (g)
T-1	21.600[b]	83.900[a]	3077[az]
T-2	26.700[ab]	83.700[a]	711[b]
T-3	31.700[a]	84.600[a]	3135[a]
T-4	23.800[b]	83.900[a]	3051[a]
T-5	27.100[ab]	85.400[a]	3076[a]
SEM	0.016	0.085	70.6
P-value	0.005	0.637	>0.001

T-1: Control diet, T-2: Control diet +0.4 ppm selenium from sodium selenite, T-3: Control diet +0.4 ppm selenium from sel-plex (commercial organic selenium), T-4: Control diet +0.4 ppm selenium from *Saccharomyces cerevisiae*-fermented rice bran, T-5: 0.4 ppm selenium from *Aspergillus niger*-fermented rice bran

Table 6: Selenium intake, selenium digestibility and meat selenium of broilers fed the experimental diets

Treatments	Selenium intake (g)	Selenium digestibility (%)	Meat selenium (ppm)
T-1	0.055[d]	65.000[a]	0.890[c]
T-2	0.175[c]	38.700[b]	1.110[bc]
T-3	0.429[b]	64.900[a]	1.400[a]
T-4	0.559[a]	66.600[a]	1.170[ab]
T-5	0.425[b]	68.700[a]	1.200[ab]
SEM	0.012	0.046	0.044
p-value	>0.001	>0.001	>0.001

T-1: Control diet, T-2: Control diet +0.4 ppm selenium from sodium selenite, T-3: Control diet +0.4 ppm selenium from sel-plex (commercial organic selenium), T-4: Control diet +0.4 ppm selenium from *Saccharomyces cerevisiae*-fermented rice bran, T-5: 1 ppm selenium from *Aspergillus niger*-fermented rice bran

crude fibre was relatively high, being 14.3% in the present study. An extra 0.1% sodium selenite in rice bran fermented by *Saccharomyces cerevisiae* and *Aspergillus niger* could increase protein concentration dramatically by 104 and 84% respectively. It is hard to determine whether the increase in protein was due to fermentation per se or combination of fermentation and addition of sodium selenite. It is possible that this is only a matter of compensating other nutrient reduction as crude fibre content decreased from 14.3% to 11.6-11.8% due to fermentation. These findings clearly indicate that both fungi could utilized fibrous fraction to be used as source of energy for fungi. A 9.6% of biomass loss found in *Saccharomyces cerevisiae*-fermented rice bran might indicate this fungi grown well as compared to Aspergillus niger with the biomass loss of 6.4%. It is well accepted that the faster their growth, the more nutrients were utilized.

Addition of commercial selenium, inorganic selenium or enriched selenium rice bran fermented by fungi in the diets increased analysed selenium content from 0.15 ppm (T-1) to 2.05 ppm (T-2), 1.16 ppm (T-3), 1.54 ppm (T-4) and 1.18 pmm (T-5). Addition of 0.1% selenium into the substrate could mathematically increased selenium content of rice bran to

1000 ppm. The fermentation either by using Aspergillus niger or *Saccharomyces cerevisiae* produced substrates with 658 and 732 ppm selenium respectively. Reduction in 342 and 268 ppm of selenium due to fermentation found in this study might indicate that sodium selenite was used and metabolized by fungi through methylation of selenite. This process released waste product of hydrogen selenite into the air and thus total concentration of selenium in the substrate decreased. Main products of selenium metabolism by fungi is the production organic selenium from inorganic sodium selenite. According to Demirci *et al.*[18] yeast *Saccharomyces cerevisiae* could produce up to 3000 µg selenomethionine from 1 g selenium. This speculation needs further investigation by determining the seleno-amino acids production.

Effects of diets on growth performance: The addition of commercial selenium as a feed additive produced by Alltech company (®Selplex) in broiler diets produced heavier birds. Sundu *et al.*[19] found that the addition of commercial selenium feed additive (Sel -plex) increased body weight gain of birds by about 20%. However, these current findings did not support the previous finding of Sundu *et al.*[19]. Body weight gain of birds fed the commercial selenium feed additive at weeks 4, 5 and 6 was unchanged. The different findings might be due to the quality of the control diet used in the study of Sundu *et al.*[19] and in the current study. This logic is based on the fact that the body weight gain of birds at week 4 of the previous study was only 858 g while body weight gain of birds fed the control diet in the present study was 1133 g. This finding might indicate that the efficacy of selenium supplementation in the diet become evident when the diet was of low quality.

Supplementation of the diets with 0.4 ppm selenium in the form of sodium selenite decreased body weight gain of broiler. This indicates that sodium selenite could not be used as a feed additive to meet the selenium requirements for monogastric animal, particularly broiler chickens. A dramatic decrease in body weight gain of birds fed the sodium selenite-supplemented diet might prove the poisonous effect of this chemical substance. Fermentation of the diet containing 0.4 ppm sodium selenite in rice bran could minimize the detrimental effect of the sodium selenite. It could be stated that there was a bioconversion on inorganic selenium of sodium selenite into organic substances in the form of selenium-containing amino acids (seleno methionine or seleno-cysteine). The bioconvertion process could possibly minimize the concentration of poisonous sodium selenite and at the same time increased the concentration of selenium-containing amino acids[18]. This speculation is based on the fact that fermentation of rice bran containing the same amount of sodium selenite could produce body weight gain of birds similar to body weight gain of the control birds and the commercial selenium-fed birds.

In the present study, feed intake of birds fed the experimental diets was dropped due to addition of sodium selenite in the diet. It has been well accepted that feed intake plays a vital role in affecting poultry performance. Reduction in growth of birds fed the diet 1.0 ppm selenium from sodium selenite was highly due to a decreased feed intake. When the feed consumption of 6-week old birds dropped by 367%, body weight gain was also dramatically deteriorated by 321%. The birds fed sodium selenite migh experience selenium toxicity. The ability of birds to discriminate toxin-containing diet is a survival mechanism for any living creature to maintain their existence. Accordingly, when the birds were offered the highly toxic selenium diet, the birds would discriminate the diets by reducing feed intake. According to Surai *et al.*[20] addition of 0.3 ppm sodium selenite in the diet could damage gut structure of broilers and degenerate vacuolar in the epithelial cells lining of gastrointestinal crypts. Feed conversion ratio of birds fed the experimental diets increased as the birds got older. Since the birds fed the 0.4 ppm sodium selenite had higher feed intake and lower body weight gain, the birds were higher in FCR than those of birds fed the other experimental diets.

Dry matter feces, dry matter digestibility and digestible dry matter intake: Dry matter feces of birds fed the experimental diets was in the range of 21.6-31.7%. The addition of selenium in the diets increased dry matter feces. This become evident as birds fed the Sel-plex-supplemented diets produced drier fecal discharges than those of bird fed the control diet. It is hard to elaborate as selenium from Selplex was the only selenium-supplemented diets that could affect fecal dry matter. As the wet feces has been an environmental issue in promoting strong aroma due to ammonia production, results of the present study could become an alternative for the environmentally friendly poultry production.

The effect of diets supplemented with selenium on dry matter digestibility has been reported by Sundu *et al.*[19], who found that supplementation of the diets with high selenium feedstuffs could not improve dry matter digestibility. The dry matter digestibility of diets, found in the current study, was in the range of 83.9-85.4%. This digestibility gap among treatment diets was statistically not significant and thus support the previous finding of Sundu *et al.*[19]. It is unexpected that addition of sodium selenite did not impair feed digestibility in the present study. It might be due to the fact that the sodium selenite-fed birds consumed too small amount of diet and thus digestive tract of broilers could optimally digest feed consumed.

Although, dry matter digestibility was not affected by experimental diet, digestible dry matter intake of birds fed the sodium selenite diet was much lower than the other experimental birds. This was much related to feed intake as digestible dry matter intake was a function of dry matter digestibility and feed intake. It is important to take into account that digestible dry matter intake is a good parameter strongly correlating to body weight gain. This current finding proved that growth of birds was linearly correlated with digestible dry matter intake ($R^2 = 0.9995$ and $Y = 0.6606X+12.027$).

Selenium intake, digestibility and meat selenium: The increase in selenium intake of birds fed the selenium-supplemented diets was much related to feed intake and selenium concentration of the diet. Data clearly indicated that when the diet was added with selenium, selenium intake was enhanced. Supplementation of the diets with selenium from yeast and rice bran with additional inorganic selenium fermented with fungi (T4 and T5) did not increase selenium digestibility. However, when sodium selenite was added into the diet without any fermentation, selenium digestibility dropped dramatically. This finding support the previous finding of Briens *et al.*[21], who stated that apparent selenium digestibility was dropped by 50% when the diet was supplemented with 0.3 ppm sodium selenite, compared to 0.3 ppm organic selenium-supplemented diet.

Efficacy of solid state fermentation using fungi in increasing nutrients profile of rice bran, copra meal, coconut flour and palm kernel meal have been reported by Hatta *et al.*[10], Sukaryana *et al.*[11] and Mozin *et al.*[22]. Sukaryana *et al.*[11] stated that fermentation could bioconvert inorganic minerals into organic materials. The current finding of increased digestibility of diets containing sodium selenite due to fermentation indicated that fermentation might convert inorganic sodium selenite (toxic selenium) into organic forms, either seleno-methionine or seleno-cysteine. This speculation was based on the fact that selenium digestibility of T4 and T5 (fermented rice bran with additional sodium selenite) produced better selenium digestibility than the control diet. This speculation needs to be proved by analysing the selenium-containing protein in the sodium selenite-added rice bran before and after fermentation.

The effect of addition of selenium in the diet on meat selenium has been reported Sundu *et al.*[19] and Briens *et al.*[21]. Sundu *et al.*[19] found that when control diet was added with commercial selenium feed additive and snail meal (*Melania testudinaria*), a high selenium feedstuff, breast muscle selenium increased. However adding the same concentration of selenium from tuna fish meal and *Moringa oliveira* seed meal could not improve breast muscle selenium. It is interestingly to state here that meat selenium found in this study did not correlate with selenium intake ($r^2 = 0.543$) and selenium digestibility ($r^2 = 0.021$). Although, there was an increase in selenium intake, meat selenium of birds fed the inorganic selenium-supplemented diet did not enhance, compared to the meat selenium of birds fed the control diet. Sundu *et al.*[19] hypothesized that the source of selenium might play a pivotal role in storing selenium in the muscle and other tissues. Inorganic selenium (sodium selenite) seemed to be unabsorable as selenium digestibility of the diet containing sodium selenite was negatively affected in this present study.

CONCLUSION

The use of 0.4 ppm sodium selenite in the diet downgraded the feeding value of diet through the decreased broiler growth performance at weeks 4, 5 and 6, selenium digestibility and meat selenium. Feeding the birds with the either *Aspergillus niger* or *Saccharomyce cerevisiae*-fermented coconut dregs increased body weight of broiler chickens, selenium digestibility and meat selenium to the same performance of birds fed the commercial selenium feed additive.

SIGNIFICANCE STATEMENT

The current study discovered the use of 0.4 ppm inorganic selenium in the form of sodium selenite could deteriorate the feeding value of the diets. Fermentation by fungy either *Saccharomyces cerevisiae* or *Aspergillus niger* could eliminate the detrimental effect of inorganic selenium when sodium selenite was used at the concentration of 0.4 ppm. The efficacy of fermentation to improve the feeding value of diets and meat selenium was as effective as using commercial selenium feed additive.

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