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Evaluation of energy and amino acids of brown rice and its effects on laying performance and egg quality of layers

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Abstract

Two experiments were conducted to determine apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), and ileal digestible amino acid (AA) content of brown rice (BR) and to investigate the effect of dietary supplementation of BR on laying performance and egg quality of laying hens. In Exp. 1, 72 Hy-line Brown layers (49-week-old) were allocated to two treatments using a completely randomized block design, and each treatment included six cages per treatment and six hens per cage. A semi-purified diet was formulated to include BR as the sole source of AA and energy and an N-free diet was used to determine basal endogenous loss of AA. The hens were fed a commercial layer diet for adaptation to the experimental environment and diet for 7 days from d 0, and then fed experimental diets for 5 days from d 7. Excreta were collected from d 10 to 11 and ileal digesta were collected on d 12. On a dry matter (DM) basis, the AME and AMEn of BR was determined at 3,773 and 3,729 kcal/kg, respectively. The apparent ileal digestibility (AID) of BR ranged from 32.7% for Thr to 73.7% for Arg. The range of the standardized ileal digestibility (SID) value was between 79.4% for Met and 96.6% for Lys. In Exp. 2, 252 Hy-line Brown layers (44-week-old) were divided into four groups, comprising seven replicates of nine birds each and assigned to four experimental diets containing 0 (Control), 5%, 10%, or 15% BR for 5 weeks. The BR-containing diets were formulated to be equal in the content of AMEn and digestible AA to those of the diet without BR. No significant differences were observed in laying performances. Egg quality and blood profiles were not linearly or quadratically affected by dietary treatments. These results suggest that up to 15% BR can be included into layer feed without any adverse effects on laying performance and egg quality, if its energy and digestible AA values are well evaluated.

Keywords: Brown rice, Apparent metabolizable energy, Standardized ileal digestible amino acids, Egg production, Laying hens

Competing interests
No potential conflict of interest relevant to this article was reported.

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: An BK, Kim KE, Kim EJ, Lee SR, Kong C.
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The Institutional Animal Care and Use Committee at Konkuk University approved the techniques and procedures involved in the animal care and handling (KU18136).

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for humans and is produced in many countries worldwide. In Korea, rice production is increasing, whereas rice consumption per capita is decreasing, resulting in an increase in rice stockpiles. Rice stored for a long time is rejected for human consumption because of its low eating quality, and is thus being supplied for feedstuff at prices competitive to those of cereal grains. As a potential feedstuff, brown rice (BR) has been used in feeding trials in poultry diets and has demonstrated similar efficiency to corn grain in egg production or growth [1,2]. Sittiya et al. [3] also reported that laying performance and egg qualities, except for yolk color, were not influenced by dietary treatment in which corn was replaced with rice at different levels. However, it has been noted that BR varieties show high variability in feeding value, especially in true metabolizable energy [4].

It is well known that information on the availability of energy and amino acids (AA) in newly applied feedstuff is necessary for more accurate diet formulation and a control on diet quality [5]. Larbier et al. [6] also suggested that diet formulation using vegetable protein feedstuffs on an available AA basis is more advantageous than formulation on a total AA basis. Therefore, BR that has been stored long-term should be evaluated as a potential feed ingredient for layers before use. This study was conducted to determine metabolizable energy and digestible amino acids in BR and to investigate its optimal feeding level for commercial layers.

Materials and Methods

Ingredient, diets, animals, and management

The BR (*Oryza sativa*) used in the present experiment was obtained from Nonghyup Feed, Seoul, Republic of Korea. The BR sample was stored for three years after harvest before being used in the experiment.

In experiment 1, a metabolism study with 49-week-old Hy-Line Brown layers was conducted for 5 days to determine the utilization of AA and energy in the BR used for the feeding trial. A semi-purified diet containing BR as the sole AA and energy source was used to determine the apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) as well as the apparent ileal digestibility (AID) of AA in the BR (Table 1). An N-free diet was also used to determine the basal endogenous losses (BEL) of AA for the correction of AID to standardized ileal digestibility (SID). All diets were supplied in mash form and chromic oxide was used as an index of indigestibility. On d 0, a total of 72 hens were weighed individually and grouped into six blocks by body weight and randomly allocated to two dietary treatments in each block with six hens per cage in a completely randomized block design. The hens were fed a commercial layer diet for adaptation to the experimental environment and diet for 7 days from d 0, and then fed experimental diets for 5 days from d 7. The diets and water were supplied *ad libitum* during the adaptation and experimental periods.

In experiment 2, a feeding trial lasting 5 weeks was conducted using 252 Hy-Line Brown layers (44-week-old). Four experimental diets were formulated to investigate optimal feeding level of BR, and 0%, 5%, 10%, and 15% of BR was incorporated into corn-soybean meal-based diets at the expense of corn and soybean meal (Table 2). Limestone addition increased with increasing levels of BR. The hens were housed in a caged layer house and had *ad libitum* access to the experimental diets and water. Hens were randomly allocated to the four experimental diets with seven replicates in a completely randomized design. Each replicate comprised three cages with three birds per wire cage (48 × 48 cm).

Table 1. Composition of ingredients in the diets used in Experiment 1; as-fed basis

Item	Experimental diets	
	Brown rice	NFD[1)]
Ingredient (%)		
Ground brown rice	94.82	-
Cornstarch	-	28.79
Sucrose	-	55.00
Soybean oil	-	4.00
Ground limestone	1.99	1.88
Monocalcium phosphate	1.79	2.14
Chromic oxide	0.50	0.50
Sodium chloride	0.40	-
Vitamin-mineral premix[2)]	0.50	1.00
Cellulose	-	5.00
Choline chloride	-	0.25
Magnesium oxide	-	0.09
Potassium carbonate	-	0.30
Potassium chloride	-	0.30
Sodium bicarbonate	-	0.75
Total	100	100

[1)]Nitrogen-free diet (NFD) contained a calculated electrolyte balance of 114.8 mEq.

[2)]Provided per kg of diet: vitamin A, 80,000 IU; vitamin D_3, 15,000 IU; vitamin E, 40 IU; vitamin K_3, 6.6 mg; thiamine nitrate, 4.85 mg; riboflavin, 25 mg; pyridoxine hydrochloride, 10 mg; vitamin B_{12}, 60 mg; nicotinic acid, 100 mg; calcium pantothenate, 40 mg; folic acid, 3 mg; choline chloride, 1,750 mg; Mn, 600 mg as manganese sulfate; Zn, 450 mg as zinc sulfate; Fe, 200 mg as ferrous sulfate and ferric oxide; Cu, 25 mg as cupric sulfate; I, 12.5 mg as calcium iodate; Co, 5 mg as cobaltous carbonate; and Se, 2.5 mg as sodium selenite.

For both experiments 1 and 2, the hens were reared under standard conditions of temperature (23℃ ± 3℃), humidity (60%), and artificial lighting (16L/8D). Vitamins and trace minerals in all diets were included to meet or exceed nutrient requirement estimates [7].

Excreta and digesta collection and chemical analyses

In experiment 1, excreta collection were performed twice a day from d 10 to 11. On d 12, all the birds were killed by CO_2 asphyxiation, and the ileal digesta samples were collected from the distal ileum through flushing with distilled water. The collected ileal samples were pooled by each cage. The collected excreta and ileal digesta samples were stored in a freezer at –20℃ until further analyses. At the end of the experiment, excreta and ileal digesta samples were thawed and the excreta samples were dried in a forced-air oven at 55℃ for 96 h and all ileal digesta samples were freeze-dried.

Gross energy (GE) of ingredient, diets, and excreta samples was determined in an isoperibol bomb calorimeter (Parr 6200, Parr Instruments, Moline, IL, USA). The experimental ingredient, diets, excreta, and ileal digesta samples were analyzed for crude protein (CP; AOAC [8]; method 990.03). The content of AA in the experimental ingredient, diets, and ileal digesta samples was determined (AOAC [8]; method 994.12; method 988.15). Chromium concentrations in the diets, excreta, and ileal digesta were analyzed according to the procedure described by Fenton and Fenton [9].

Egg production

In experiment 2, egg production and egg weight were determined daily. Except for abnormal eggs,

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Table 2. Ingredients and calculated chemical composition of diets used in Experiment 2; as-fed basis

Ingredients (%)	Brown rice (%)			
	0	5	10	15
Corn	53.55	48.75	43.85	38.85
Wheat	5.00	5.00	5.00	5.00
Brown rice	0.00	5.00	10.00	15.00
Soybean meal	15.70	15.50	15.30	15.10
Canola meal	4.00	4.00	4.00	4.00
DDGS	5.00	5.00	5.00	5.00
Corn gluten meal	4.00	4.00	4.00	4.00
Tallow	1.30	1.20	1.20	1.30
Lysine HCl, 78%	0.20	0.20	0.20	0.20
DL-Met, 98%	0.10	0.10	0.10	0.10
Monocalcium phosphate	0.60	0.60	0.60	0.60
Limestone	10.00	10.10	10.20	10.30
Salt	0.30	0.30	0.30	0.30
Vitamin mixture[1)]	0.10	0.10	0.10	0.10
Mineral mixture[2)]	0.10	0.10	0.10	0.10
Phytase	0.05	0.05	0.05	0.05
Total	100.0	100.0	100.0	100.0
Calculated nutrient content				
AMEn (kcal/kg)	2,700	2,700	2,700	2,700
Crude protein	17.0	17.0	17.0	17.0
Ca	4.1	4.1	4.1	4.1
Non phytate P	0.4	0.4	0.4	0.4
SID amino acid				
Arg	0.88	0.88	0.88	0.88
His	0.40	0.39	0.38	0.38
Ile	0.62	0.61	0.60	0.59
Leu	1.54	1.52	1.49	1.46
Lys	0.65	0.65	0.64	0.63
Met	0.28	0.28	0.28	0.27
Cys	0.25	0.25	0.25	0.24
Phe	0.77	0.76	0.75	0.74
Thr	0.57	0.56	0.55	0.54
Trp	0.14	0.14	0.13	0.13
Val	0.70	0.69	0.69	0.68

[1)]Vitamin mixture provided following nutrients per kg of diet: vitamin A, 40,000 IU; vitamin D_3, 8,000 IU; vitamin E, 10 IU; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 20 mg; folic acid, 2 mg; nicotinic acid, 60 mg.

[2)]Mineral mixture provided following nutrients per kg of diet: Fe, 60 mg; Zn, 50 mg; Mn, 25 mg; Cu, 10 mg; I, 1.7 mg; Se, 0.10 mg.

DDGS, distiller's dried grains with solubles; AMEn, nitrogen-corrected apparent metabolizable energy; SID, standardized ileal digestible.

replicated egg weight was measured for daily average of egg weight. Egg mass was calculated by multiplying the egg weight by the egg production. At the beginning and the completion of the experiment, feed intake was measured, and average daily feed intake and egg production were corrected with mortality.

Egg quality measurements

Egg quality was determined at 14 and 28 d of feeding. Five eggs from each replicate were collected, weighed and stored overnight at room temperature (23℃ ± 3℃) for subsequent analyses. Albumen height was measured using the DET-6000 Egg multi-tester (Nabel, Japan). The Haugh unit calculation was performed as previously described by An et al. [10]. The breaking strength for uncracked eggs was determined using an eggshell strength tester (Nabel, Japan). Eggshell thickness without shell membrane was measured using a micrometer (Digimatic micrometer, Series 547-360, Mitutoyo, Japan). Egg yolk color was determined by comparison with the Roche yolk color fan (Hoffman-La Roche, Basel, Switzerland).

Blood sampling and analysis

At the completion of the experiment, blood samples from the jugular vein were collected from seven birds per treatment. After centrifugation at 2,000×g for 15 min, the serum samples were stored at –60℃ until analysis. The activity of glutamic oxaloacetic transaminase (GOT) in sampled sera was measured using the colorimetric method using GOT assay kits (Asan Pharmaceutical, Korea) according to the manufacturer's instructions. The levels of serum total protein, globulin, and albumin were measured using an automatic blood analyzer (Labospect 008AS, Hitachi, Japan).

Statistical analysis

All the data obtained in Exp. 2 were analyzed using the GLM procedure in SAS [11]. Orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of dietary BR on the responses measured. Statistical significance was accepted at $p < 0.05$.

RESULTS

The analyzed chemical compositions of BR used in experiments 1 and 2 are presented in Table 3. On an as-fed basis, the CP and GE content of BR was 8.83% and 3,854 kcal/kg, respectively. The concentration of AA ranged from 0.185% for Cys to 1.457% for Glu.

Experiment 1

The data presented in Table 4 show the apparent total tract retention of GE and AMEn of the BR fed to laying hens. On a dry matter (DM) basis, the AME and AMEn of BR was determined as 3,773 and 3,729 kcal/kg, respectively. Basal ileal endogenous losses of AA determined from laying hens fed an N-free diet ranged from 334 mg/kg DMI for Met to 2,571 mg/kg DM intake (DMI) for Glu (Table 5). Table 6 shows the AID and SID of AA of BR determined at the terminal ileum of laying hens. The AID of BR ranged from 32.7% for Thr to 73.7% for Arg. The range of SID values was between 79.4% for Met and 96.6% for Lys.

Experiment 2

The laying performance of hens fed diets with varying BR levels is presented in Table 7. Feed intake, egg production, egg weight, and daily egg mass were not affected by increasing level of BR in the diets. The results presented in Table 8 show that increasing levels of BR did not influence any egg quality parameters. The effects of varying levels of dietary BR on the blood profiles are summarized in Table 9. The serum total protein, globulin, albumin, and GOT concentration did not change as dietary BR concentration increased.

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Table 3. Analyzed chemical composition of brown rice and experimental diet fed to laying hens in Experiment 1; as-fed basis

Item (%)	Brown rice	Experimental diet
Dry matter	87.81	89.94
Gross energy (kcal/kg)	3,854	3,216
Crude protein	8.83	7.65
Indispensable amino acid		
Arg	0.642	0.550
His	0.201	0.171
Ile	0.321	0.280
Leu	0.718	0.619
Lys	0.368	0.314
Met	0.186	0.157
Phe	0.448	0.392
Thr	0.334	0.294
Val	0.445	0.394
Dispensable amino acid		
Ala	0.479	0.414
Asp	0.746	0.645
Cys	0.185	0.159
Glu	1.457	1.267
Gly	0.378	0.325
Pro	0.472	0.435
Ser	0.430	0.381
Tyr	0.364	0.344

Table 4. Apparent total tract retention (ATTR) of energy in brown rice fed to laying hens, DM basis[1)]

Item	Mean	SD
ATTR of GE of diet (%)	86.0	2.29
AME of the brown rice (kcal/kg)	3,773	100.4
Retained N (g/kg Intake)	5.4	1.56
AMEn of the brown rice (kcal/kg)	3,729	90.7

[1)]Values are means of six replicate cages with three hens per cage.

DM, dry matter; SD, standard deviation; GE, gross energy; AME, apparent metabolizable energy; AMEn, nitrogen-corrected AME.

DISCUSSION

In Exp. 1, the GE content was in agreement with the values (3,831 and 3,800 kcal/kg on an as-fed basis) reported by Asyifah et al. [4] but was much greater than the value (3,248 kcal/kg on an as-fed basis) reported by Kim et al. [12]. The reason for this discrepancy is unclear. In the present study, a direct method in which the experimental diet included the test ingredient as the sole source of energy, was used to determine AME and AMEn of BR [13]. To our knowledge, this is the first study evaluating the AME of BR-fed laying hens. It is difficult to directly compare the AME determined in the present study and values determined in a previous study. However, the AME of 3,773 kcal/kg DM is the value between the true metabolizable energy values (3,310 and 4,200 kcal/kg DM) of two BR samples measured by Asyifah et al. [4]. The difference in energy value between the two

Table 5. Ileal basal endogenous losses (BEL) of amino acid in laying hens fed a nitrogen-free diet[1)]

Item	BEL (mg/kg dry matter intake)
Indispensable amino acid	
Arg	1,138
His	492
Ile	957
Leu	1,735
Lys	1,125
Met	334
Phe	879
Thr	1,527
Val	1,084
Dispensable amino acid	
Ala	1,080
Asp	2,015
Cys	715
Glu	2,571
Gly	1,108
Pro	1,084
Ser	1,476
Tyr	838

[1)]Values are means of five replicate cages with six hens per cage.

Table 6. Apparent and standardized ileal digestibility (%) of amino acids in brown rice fed to laying hens[1)]

Item (%)	Apparent ileal digestibility		Standardized ileal digestibility	
	Mean	SD	Mean	SD
Indispensable amino acids				
Arg	73.7	8.0	94.4	8.1
His	62.2	11.9	91.0	12.2
Ile	53.6	14.2	87.8	14.7
Leu	60.0	12.6	88.0	12.7
Lys	60.9	12.9	96.6	14.1
Met	58.1	15.8	79.4	13.4
Phe	66.4	10.4	88.8	9.9
Thr	32.7	21.1	84.6	23.3
Val	61.4	11.7	89.0	11.7
Dispensable amino acids				
Ala	61.5	12.4	87.6	11.2
Asp	57.1	13.3	88.4	13.0
Cys	37.0	19.3	82.1	20.7
Glu	68.9	10.0	89.2	8.4
Gly	54.4	13.6	88.6	14.0
Pro	66.4	10.6	91.3	11.4
Ser	49.1	16.0	87.8	16.6
Tyr	63.8	10.9	88.1	10.0

[1)]Values are means of six replicate cages with six hens per cage.

SD, standard deviation.

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Table 7. Effect of graded levels of brown rice on egg laying performance in laying hens[1)]

Item	Brown rice (%)				SEM	*p*-value	
	0	5	10	15		Linear	Quadratic
Feed intake (g/bird/d)	113.4	112.6	114.1	114.4	1.75	0.563	0.776
Egg production (%)	88.0	92.1	91.6	91.2	2.65	0.443	0.416
Egg weight (g/egg)	61.9	61.5	61.7	61.8	0.52	0.976	0.654
Daily egg mass	54.4	56.5	56.5	56.3	1.26	0.430	0.486

[1)]Data are least square of mean of seven replicates with three cages (three birds per cage). Mean values from the overall experimental period.

SEM, standard error of the mean.

Table 8. Effect of graded levels of brown rice on egg qualities in laying hens[1)]

Item	Brown rice (%)				SEM	*p*-value	
	0	5	10	15		Linear	Quadratic
Yolk color, RCF	7.1	7.0	7.2	7.2	0.08	0.219	0.620
Eggshell strength (kg/cm^2)	4.9	4.8	4.9	4.9	0.11	0.908	0.411
Eggshell thickness, 0.01 mm	38.1	37.1	37.4	37.6	0.39	0.420	0.157
Haugh unit	87.3	87.3	86.8	86.6	0.95	0.547	0.895

[1)]Data are least square of mean of seven replicates with three cages (three birds per cage). Mean values from the 2nd and 4th week after feeding.

SEM, standard error of the mean; RCF, Roche color fan.

Table 9. Effect of graded levels of brown rice on blood profiles in laying hens[1)]

Item	Brown rice (%)				SEM	*p*-value	
	0	5	10	15		Linear	Quadratic
Total protein (g/dl)	5.6	5.7	5.9	6.1	0.20	0.083	0.654
Globulin (g/dl)	3.2	3.2	3.4	3.6	0.17	0.115	0.554
Albumin (g/dl)	2.4	2.4	2.5	2.5	0.05	0.071	0.891
GOT (U/L)	166.3	175.4	173.7	174.3	7.66	0.524	0.583

[1)]Data are least square of mean of seven birds per treatment.

SEM, standard error of the mean; GOT, glutamic oxaloacetic transaminase.

studies may, in part, be attributed to the method for evaluation as well as the composition of samples used in these studies. In poultry diets, grains such as corn and BR are mainly used as energy yielding ingredients. Barzegar et al. [14] determined GE and AME values of corn using 42-week-old laying hens as 3,920 (as-fed basis) and 3,791 kcal/kg (DM basis), respectively. Those values were comparable with the values (3,854 kcal/kg for GE and 3,773 kcal/kg for AME) of BR used in the present study, which may indicate that BR has the potential to be used as an energy source in poultry diet.

Amino acids play an important role in the growth of structural and protective tissues as well as metabolic functions in animals. Although the protein content of cereal grains such as corn and BR is relatively lower than that of the protein supplements, the grain accounts for a great proportion of the mixed feed; therefore, the protein supplied by the grain is significant. Thus, evaluating protein quality such as AA bioavailability or digestibility is needed to provide the accurate amount of AA required by poultry for normal functions. It has been generally accepted that SID is a better estimate than AID for formulating poultry diets because SID corrects for BEL to accurately reflect AA needs of the birds [15]. Similar to AME data, there is no available data for digestibility of AA in BR for comparison with the values derived from the present study. As the BR could be used as a replacement for corn, it would be reasonable to compare BR in the present study with corn in other studies. The SID of indispensable AA in BR from the present study was slightly less than that in corn reported by Adedokun et al. [15] except for Arg, His, Lys, and Val. However, the SID of most AA, except Met, was 85% or more and the difference in average SID of total AA was only 1 percentage point (89% for corn vs. 88% for BR), which may indicate that BR could replace corn as an AA source.

In the present study, the differences between AID and SID were slightly great and ranged from 24.8 (Glu) to 51.8 (Thr) percentage points. The reasons for the large difference between the digestibility of the two might be the combined effect of the high amount of BEL of AA as well as the low amount of AA in BR. In the present study, the BEL of AA of laying hens was greater than that obtained in the study by Adedokun et al. [16] but it was less than values reported by Adedokun et al. [17]. The reason for this is not clear, but it can be speculated that the difference might be partly attributed to the age of the hens as well as diet composition of the nitrogen-free diet [18,19]. There were differences in calcium, starch, and sugar levels between the present and previous studies and these might contribute to the difference in the BEL [16].

In Exp. 2, the overall laying performance was not influenced by dietary BR levels, which was consistent with findings of a previous study. Sittiya et al. [3] observed that various levels of dietary paddy rice did not affect feed intake and egg production when nutrient levels were maintained in the experimental diets. Dietary BR as a replacement for corn led to a similar laying performance as that of the control diet without BR, which means that BR can be safely used up to 15% in layer diets. The AMEn value (3,273 kcal/kg) of BR derived from Exp. 1 was considered to be appropriate because there was no difference in feed intake among treatments.

There was no significant linear or quadratic trend of dietary levels of BR affecting egg and eggshell qualities. In contrast, Sittiya et al. [3] reported that yolk color was significantly decreased with increasing levels of paddy rice (50% or more) without affecting other egg qualities. This negative effect can be attributed to low levels of xanthophyll in rice along with the presence of other feedstuff affecting yolk color. In this study, corn gluten meal and distiller's dried grains with solubles were included at a 4% or 5% level, respectively, and there was no influence on yolk color. In addition, supplementation of BR at the 15% level did not affect yolk color.

No significant differences were found in the activity of serum GOT, or in the content of albumin, globulin, and total protein among the groups. Similar to our results, no significant effect of rice feeding was found on serum biochemical parameters of broiler chickens [20]. Blood profiles could be used as general evidence to evaluate the health status of animals. Serum GOT activity with which it is possible to diagnose liver or tissue damage, is a valuable tool to estimate the safe levels for alternative feed ingredients [21]. The levels of serum albumin and globulin are indicator for hepatocyte injury and immune response, respectively [22,23] and they can also be nutritional status indicator [24]. The result for blood profiles from the present study may indicate that there are no detrimental health and nutritional impacts of BR fed to layers up to 15% in diet.

Overall, we did not find any significant effects of replacing corn with BR on egg production, egg quality, and blood profiles. These results indicate that BR can be included into layer diets up to 15% without any adverse effects, if its energy and digestible AA values are well evaluated.

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