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Effect of Dietary β-Mannanase Supplementation and Palm Kernel Meal Inclusion on Laying Performance and Egg Quality in 73 Weeks Old Hens

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ABSTRACT

This study was conducted to evaluate the effect of dietary β-mannanase supplementation and palm kernel meal (PKM) inclusion (5%) on laying performance, egg quality and nutrient utilizability of laying hens with 73 weeks of age. A total of 240 Lohmann brown laying hens with average 77.5% egg production were randomly allocated with 60 hens per treatment, 4 replicates per treatment and 15 hens per replicate. Experimental design was a completely randomized design with 2 × 2 factorial arrangement, with the factors being (1) two levels of PKM (0 vs. 5%) and (2) with or without dietary β-mannanase (480 IU/kg of diet CTCzyme®) supplementation. All hens were housed in cages (35 cmW × 35 cmD × 40 cmH) with 2 hens per cage for six weeks feeding trial. Laying performance was recorded daily during feeding trial. Egg quality, nutrients utilizability and blood assays were done at the end of feeding trial. Egg production was improved ($P<0.05$) by both dietary PKM inclusion and β-mannanase combined supplementation. Either β-mannanase or PKM did not affect feed intakes and feed conversion ratio of all diets. Egg weight of hens fed diet containing 5% of PKM had heavier ($P<0.05$) eggs compared with hens fed without PKM. Albumen height was improved ($P<0.05$) by dietary mannanase supplementation. Crude fat utilization of 5% PKM diet was higher than that of no PKM diet regardless of β-mannanase supplementation. Both DM and total carbohydrate utilization were decreased ($P<0.05$) in hens fed 5% PKM diet. Serum IgG and yolk IgY contents of PKM groups were lower ($P<0.05$) than those of no PKM groups. This result showed that 5% PKM diet, independent of dietary β-mannanase supplementation, was able to improve egg production. In addition, dietary β-mannanase supplementation could be used for improving the albumen height of eggs.

(**Key words :** β-mannanase, 73 weeks old laying hen, Palm kernel meal, Laying performance, Egg quality)

INTRODUCTION

Old aged laying hens are known to consume less feed with increasing age (Pavlik et al., 2009), which has led to a decrease in egg production. Therefore, the egg production from the hens older than 65 ~ 70 weeks has always been a serious challenge especially on economic stand point. Recently, the profit of egg production against the cost of feed became even worse due to the price hike of feed ingredients. The decrease in feed consumption and following decline in egg production in old aged hens could be attributed to the decreased metabolic rate in the old aged hens compare to young hens (Lin et al., 2006; Pereira et al., 2010). To recover the disadvantage of old aged hens, producers have sought the ways to reduce feed cost as well as to enhance metabolic efficiency in the old aged hens.

To reduce the cost of feed, inclusion of relatively cheaper ingredient such as palm kernel meal (PKM) has been proposed. PKM contains average 14 ~ 16% CP and 1,600 ~ 1,700 kcal ME, for chickens. But the PKM also contains 35 ~ 40% mannans, which is poorly digestible by poultry (Sundu et al., 2006). Therefore, supplementation of its respective enzyme, mannanase has been recommended (Wu et al., 2005; Sundu et al., 2006). In addition, several poultry studies (Sundu et al., 2006; Chong et al., 2008; Zanu et al., 2012) showed that the appropriate level of PKM inclusion had encouraged the feed intake in laying hens. Therefore, it was hypothesized that the PKM might act as a stimulator of feed intake in the old aged hens.

However, little information is known how dietary PKM inclusion and mannanase supplementation could affect old aged laying hens in terms of egg production performance, nutrient utilization and egg quality parameters. Therefore, this study was conducted to investigate the effect of β-mannanase supplementation to PKM included diet on laying performance, egg and shell quality, nutrients utilization and

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immune response in old aged laying hens.

MATERIALS AND METHODS

The protocols for feeding trials, nutrients utilization assay and blood collection methods were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Korea.

1. Experimental laying hens and care of the birds

A total of 240 Lohmann brown laying hens of 73 weeks of age with average 77.5% egg production were randomly allocated into 4 treatments with 4 replicates per treatment and 15 hens per replicate. The 4 treatments were arranged in a 2 × 2 factorial design with two levels (0 IU vs. 480 IU / kg of diet) of dietary β-mannanase and two levels (No vs 5%) of dietary palm kernel meal (PKM) inclusion. β-mannanase used in this study was CTCzyme® (CTCBio Inc., Korea) containing 800,000 unit β-mannanase/kg supplement. All the birds were allocated to 2 floor straight type steel wire laying hen cages with 2 hens per each cage room (35 cmW × 35 cmD × 40 cmH) for six weeks. At the end of feeding trial, 5 laying hens with similar body weight and egg production were chosen from each treatment. Totally, 20 laying hens were individually housed on A-type steel wire laying hen cage for nutrient utilization assay. After 5 days of acclimatization, 100 g of each experimental diet was offered to each hen per day for 3 consecutive days. At the first and the last day, daily feeds were mixed with 0.3% ferric oxide as a marker for fecal collection.

The 15L: 9D (15lux/m^2 of illumination) lighting program was implemented throughout the feeding and nutrient utilization trials. Temperature and relative humidity of hen house were maintained at 24 ~ 27ºC and 70 ~ 75%, respectively. All laying hens were vaccinated according to the conventional vaccination program. The experimental hens were debeaked in the early age of life.

During 6 weeks feeding trial, both feed and water were offered *ad libitum*. Feed was offered on plastic straight groove feeder and water was supplied by water nipple. Experimental diets were formulated to be iso-nitrogenous (16.2% CP) and iso-caloric (2,720 kcal ME/kg of diet). Corn, soybean meal (44%) and rice bran were the major ingredients although synthetic amino acids were employed to meet the amino acid requirement (Lohmann Brown Management Guide, 2007. Lohmann GB Ltd, UK). Palm kernel meal (PKM) used in this experiment was imported from South Asia, and then purchased from local feed mill in Korea. Formula and nutrients composition of experimental diets are shown in Table 1.

2. Measured items and analyses

Egg production was recorded daily at 09:00 h then egg weight and mortality were also recorded daily. Hen day egg production (HDEP), average daily feed intake (ADFI), feed conversion ratio (FCR), and mortality were calculated weekly and at the end of feeding trial. For nutrient utilization assay, the fresh excreta were collected daily from the time of first appearance of colored excreta until the time just before the reappearance of colored excreta. Freshly collected excreta were dried in an electric oven with forced aeration at 65°C until reached to the constant weight. Whole dried excreta samples were pooled, weighed, and ground then kept at room temperature for further chemical analyses. Total feed intake per hen was recorded and representative feed samples were ground for chemical analyses.

Thirty eggs per replicate were randomly collected at wk 4 of feeding trial period for the analyses of egg quality parameters. Eggshell strength was measured using egg shell intensity tester (FHK, Fujihira Industry Co., LTD, Japan). Eggshell thickness was determined using a dial pipe gauge according to the procedure (Monira et al., 2003). Albumen height was measured using tripod micrometer. Egg yolk color score was examined using the Roche 15-point color fan.

All proximate components (DM, CP, energy, ash and fat) in both excreta and diet samples were analyzed according to the standard methods of AOAC (2005). Nutrient utilization was calculated by subtracting amounts of nutrient in excreta from the amount of nutrients in consumed feed. To determine the concentration of IgY, the water-soluble fraction (WSF) of egg yolk was separated according to the methods described by Bizhanov et al. (2004), and the WSF was used to quantify IgY concentration by the protocol of ELISA kit (E30-104) as described by Lee et al. (2002) using ELISA reader (Power Wave XS, BioTek, USA). The concentration of IgY and standard antibody were measured at absorbance of 450 nm, and the test repeated three times and the mean values of optical density were obtained. Each sample was tested in triplicate and the IgY content of egg yolk was

Table 1. Formula and chemical composition of experimental feeds

PKM / β-mannanase	No PKM		5% PKM	
	0 IU	480 IU	0 IU	480 IU
Ingredients, %				
Corn	52.15	52.15	48.10	48.10
Soybean meal (44%)	18.00	18.00	17.05	17.05
Corn gluten	0.50	0.50	0.50	0.50
Meat and bone meal	4.55	4.55	4.55	4.55
Rice bran	10.16	10.16	10.16	10.16
Rapeseed meal	2.00	2.00	2.00	2.00
PKM	0.00	0.00	5.00	5.00
DDGS	2.00	2.00	2.00	2.00
Tallow	1.00	1.00	1.00	1.00
Lysine (78%)	0.11	0.11	0.11	0.11
Methionine (100%)	0.19	0.19	0.19	0.19
Dicalcium phosphate	0.34	0.34	0.34	0.34
Limestone	8.20	8.20	8.20	8.20
Salt	0.29	0.29	0.29	0.29
Sodium carbonate	0.01	0.01	0.01	0.01
Choline chloride	0.08	0.08	0.08	0.08
Antioxidant	0.01	0.01	0.01	0.01
Vit. Mix.[1)]	0.03	0.03	0.03	0.03
Min. Mix.[2)]	0.15	0.15	0.15	0.15
Phytase	0.03	0.03	0.03	0.03
Toxin binder	0.20	0.20	0.20	0.20
β-mannanase, IU/kg[3)]	0	480	0	480
Total	100.00	100.00	100.00	100.00
Chemical composition, calculated				
C. Protein, %	16.20	16.20	16.20	16.20
C. Fat, %	4.50	4.50	4.50	4.50
C. Fiber, %	3.60	3.60	3.60	3.60
C. Ash, %	15.70	15.70	15.70	15.70
Ca, %	4.00	4.00	4.00	4.00
Total P, %	1.20	1.20	1.20	1.20
Available P, %	1.00	1.00	1.00	1.00
ME, kcal/kg	2,720	2,720	2,720	2,720

[1)] The vitamin premix contains the followings per kg of diet: Vit. A, 18.000 IU; vit. D3, 4.500 IU; vit. E, 31.5 IU; menadione, 3.6 mg; thiamin, 1.8 mg riboflavin, 4.8 mg; pyridoxine, 3.6 mg; cobalamin, 0.03 mg; niacin, 22.5 mg; panthothenic acid, 15 mg; folic acid, 0.45 mg.

[2)] The mineral premix contains the followings per kg of diet: Mn, 86.4 mg; Zn, 72 mg; Fe, 74.6 mg; Cu, 6 mg; I, 1.5 mg; Co, 0.288 mg; Se, 0.216 mg.

[3)] Mannanase: CTCzyme® (CTCBio Inc., Korea) containing 800,000 unit β-mannanase / kg supplement.

Abbreviations: PKM: Palm kernel meal, DDGS: Distillers dried grain and solubles, ME: Metabolizable energy.

expressed in mg/ml of egg yolk. At the termination of feeding trial, 6 hens∨representing average body weight and egg production were selected from each treatment then fasted 24 hours. About 4 ml of blood were collected from brachial vein using vacutainer tube. The tube was settled 30 minutes at room temperature, and then∨centrifuged at 1,500 g for 10 minutes. Top liquid portion was collected as serum, and then stored in the freezer at −70ºC until analysis. Chicken IgG

ELISA Quantitation Kit (Bethyl laboratories, Inc., U.S.A) was employed for IgG analysis by reading absorbance using ELISA reader (Power Wave XS, BioTek, USA).

All the data were analyzed by SAS program (SAS Institute Inc., 2004) using the General Linear Model (GLM) procedure of a factorial ∨ design. Mean values of each replicate were represented as an experimental unit and P-values were given and then statistically significant difference among treatments at $P < 0.05$ were expressed by different superscripts.

RESULTS

HDEP was improved by dietary PKM inclusion ($P < 0.05$) as shown in Table 2. β-mannanase supplementation into PKM included diet tended ($P = 0.051$) to improve the HDEP. However, there was no interaction effect by combination of 5% palm kernel meal inclusion and β-mannanase supplementation. Either β-mannanase or PKM did not affect feed intakes although the feed intake of mannanase supplemented 5% PKM diet was numerically highest among all treatments. The highest feed intake of the mannanase supplemented 5% PKM diet resulted in highest egg production in hens fed the diet. Although the feed conversion ratio (feed/egg) of hens fed no mannanase, 5% PKM diet was lower ($P<0.05$) than that fed no mannanase, no PKM diet, there were no significant main effect from either PKM or mannanase. FCR of no mannanase, 5% PKM diet was the most efficient compared to that of other three diets.

Egg weight of mannanase supplemented 5% PKM group was significantly ($P<0.05$) heavier than that of other groups as shown in Table 3. Egg albumen height of egg from mannanase supplemented groups were higher ($P = 0.004$) than those from no mannanase groups. The albumen height of eggs from no PKM, no mannanase group was lower ($P < 0.05$) than that of other groups. There were no differences among treatments in egg shell thickness, egg shell strength and yolk color score.

Nutrients utilization of the experimental diets is shown in Table 4. DM, energy and total carbohydrate utilization of PKM diets were poorer ($P<0.01$) than those of no PKM diets. However, crude fat utilizations of PKM diets were higher ($P<0.01$) than those of no PKM diets, regardless of mannanase supplementation. Dietary mannanase supplementation

Table 2. Effect of dietary β-mannanase supplementation and palm kernel meal inclusion on egg production performance of old aged laying hens

PKM	No PKM		5% PKM		SE	Main effect (P values)		
β-mannanase	0 IU	480 IU	0 IU	480 IU		PKM	Mann	PKM* Mann
Average daily feed intake (g/hen/day)	108.37	109.99	108.23	115.60	5.67	NS	NS	NS
HDEP (%)	77.33^{b}	78.81^{b}	80.14^{ab}	83.37^{a}	2.49	0.004	0.051	NS
FCR (feed, g/egg, g)	2.11	2.10	2.03	2.09	0.10	NS	NS	NS

Abbreviations: PKM, palm kernel meal; HDEP, Hen day egg production; Mann, β-mannanase; SE, Standard error. FCR; Feed conversion ratio

[ab] Mean values with different superscript within the same row differ significantly ($P<0.05$).

Table 3. Effect of dietary β-mannanase supplementation and palm kernel meal inclusion on egg and shell quality in old aged laying hens

PKM	0 PKM		5% PKM		SE	Main effects (P values)		
β-mannanase	0 IU	480 IU	0 IU	480 IU		PKM	Mann	PKM* Mann
Egg weight (g/egg)	64.7^{b}	65.1^{b}	65.8^{b}	69.0^{a}	4.3	0.030	NS	NS
Egg shell strength (kg/cm^2)	2.76	2.40	2.45	2.53	0.67	NS	NS	NS
Albumen height (mm)	6.3^{b}	7.6^{a}	7.1^{a}	7.6^{a}	1.3	NS	0.004	NS
Yolk color score	7.9	8.0	7.9	8.1	0.6	NS	NS	NS
Egg shell thickness (μm)	350	330	330	330	5	NS	NS	NS

Abbreviations: PKM, palm kernel meal; SEM, Standard error; Mann, β-mannanase.

[ab] Mean values with different superscript within the same row differ significantly ($P<0.05$).

Table 4. Effect of dietary β-mannanase supplementation and palm kernel meal inclusion on nutrients utilization in old aged laying hens

PKM	No PKM		5% PKM		SE	Main effects (P values)		
β-mannanase	0 IU	480 IU	0 IU	480 IU		PKM	Mann	PKM* Mann
Dry matter	71.1[a]	71.7[a]	67.4[b]	68.4[b]	2.1	0.006	NS	NS
Crude protein	41.3	42.6	44.4	36.1	6.0	NS	NS	NS
Crude fat	83.9[b]	85.1[b]	89.1[a]	89.9[a]	2.6	0.003	NS	NS
Crude ash	36.6[a]	25.4[b]	35.6[a]	18.5[b]	6.2	NS	0.001	NS
Energy	74.8[ab]	75.2[a]	71.9[b]	73.2[ab]	1.9	0.021	NS	NS
Total-CHO	78.5[a]	79.5[a]	73.9[b]	75.4[b]	1.8	0.001	NS	NS

Abbreviations: PKM, palm kernel meal; SE, Standard error; Mann, β-mannanase; CHO, Carbohydrates.
[ab] Mean values with different superscript within the same row differ significantly (P<0.05).

Table 5. Effect of dietary β-mannanase supplementation and palm kernel meal inclusion on serum IgG and yolk IgY concentration in old aged laying hens

PKM	No PKM		5% PKM		SE	Main effect (P values)		
β-mannanase	0 IU	480 IU	0 IU	480 IU		PKM	Mann	PKM* Mann
Serum IgG (mg/ml)	10.65[a]	11.29[a]	10.57[a]	8.10[b]	1.98	0.057	NS	0.069
Yolk IgY (mg/ml)	12.67[ab]	13.04[a]	10.52[bc]	9.36[c]	1.84	0.001	NS	NS

Abbreviations: PKM, palm kernel meal; SE, Standard error; Mann, β-mannanase; Ig, Immunoglobulin.
[ab] Mean values with different superscript within the same row differ significantly (P<0.05).

decreased (P<0.01) crude ash utilization compared to that of no mannanase groups. There was no interaction effect between PKM and mannanase on nutrients utilization.

Both serum IgG and yolk IgY concentrations tended to be affected (P = 0.057) by dietary PKM inclusion as shown in Table 5. Both serum IgG and yolk IgY concentrations were the lowest (P < 0.05) in mannanase supplemented PKM inclusion group. Contrastingly, the serum IgG and yolk IgY concentrations were the highest (P < 0.05) in mannanase supplemented no PKM inclusion group. The higher concentrations in serum IgG proportionally exerted the higher concentrations in yolk IgY.

DISCUSSION

Egg production of old aged hens is known to be strongly associated with feed intake (Świątkiewicz et al., 2010; Catli et al., 2012). In this study, the highest HDEP in mannanase supplemented 5% PKM diet was respectively associated with the highest feed intake of the diet. It is well known that feed intake of laying hen typically decreased after 50th week (Pavlik et al., 2009) of age. Therefore, it is believed that the PKM inclusion contributed, in certain extent, for stimulating feed intake in this study. Several laying hen studies (Perez et al., 2000; Chong et al., 2008) reported increased feed intake by PKM inclusion in diets. However, there was a report (Zanu et al., 2012) which showed no difference in feed intake by dietary PKM inclusion.

It is still not proved what component in PKM could encourage the feed intake. Low water holding capacity and higher bulkiness of PKM were suggested as an accelerator of digesta passage and therefore, increased the feed intake (Sundu et al., 2006). Since the PKM inclusion level in this study was relatively low, it could be difficult to expect such a remarkable contribution to ∨ digesta passage. Medium to long chain (C12 ~ C14) fatty acids in palm kernel oil has been suggested as a taste enhancer (Rahman et al., 2010). Dietary acids were also known to impart beneficial influence on egg production of 54 ~ 70 week old hens (Soltan, 2008). Therefore, it is worth to behold the fact that the PKM typically contained 7 ~ 8% fat and about 50 ~ 60% of the fat are C12 ~ C14 fatty acids (Sahidi, 2005).

However, feed intake responses to the PKM diets in this study were bidirectional. There was no such an increase in feed intake when there was no simultaneous supplementation of mannanase. Non starch polysaccharides (NSP) including

mannan was known to increase digesta viscosity and to reduce feed intake in poultry (Van Krimpen et al., 2011). It is typical that the PKM contained 40 ~ 45% and 28 ~ 32% of NSP and mannan respectively (Sundu et al., 2006). Once the dietary mannanase had hydrolyzed the mannan, however, the magnitude of viscosity increase would be negligible as shown in Mehri et al. (2010). In this case, the appetite stimulation by PKM could not be negated by viscosity problems. This could explain why there were the bidirectional responses against PKM diets.

Both PKM and mannanase could not significantly influence FCR although the FCR of no mannanase PKM diet was lowest among treatments, only better than that of no mannanase no PKM diet. Within the same PKM diets, higher intake of the mannanase supplemented PKM diet could be a cause of the slightly poorer FCR. In this study, the mannanase supplementation did not induce any significant impact on FCR. This could be explained by the relatively lower level of mannan in the final diet. If the amount of mannan is under any impact threshold level, the benefit by respective mannanase supplementation could be also negligible.

Both PKM inclusion and mannanase supplementation exerted heavier eggs than other diets. Egg weight is certainly depended upon sufficient and balanced feed intake (Li et al., 2011). In this study, the heaviest egg weight was also observed in the group that had highest feed intake. There was a significant effect of PKM inclusion on egg weight and as previously confirmed in this study, the PKM acted as a feed intake stimulator. Dietary mannanase supplementation, regardless of PKM inclusion, increased egg albumen height. It is not clear how and why the mannanase was able to increase the albumen height. Albumen height typically depended upon feed quality and age of hens (Roberts and Ball, 2004). Poor quality fat and less available amino acids in PKM could be a cause of thinner albumen height. It is previously reported that poor quality protein and heat damaged amino acids decreased the height of egg albumen (Teleun et al., 2008). If the mannanase supplementation was able to improve the nutritional status of old aged hens, it could be postulated that the improved nutritional status could increase albumen height. Nevertheless, the average albumen height in this study was lower than typical albumen height of eggs laid at 70 weeks of age (Roberts and Ball, 2004).

There were no differences in egg shell quality due to either dietary mannanase or PKM inclusion. Shell quality including thickness and strength were known to decrease with age of hens, probably due to poorer functioning on calcium metabolism (Zita et al., 2012). Bone (Moreki et al., 2011) and serum calcium (Garlich et al., 1984) status that represent calcium metabolism were also diminished with increasing age. Therefore, replacement of poor quality calcium source with better source (Catli et al., 2012) and acidic modulation of gut by feed additives (Świątkiewicz et al., 2010; Hanafy, 2010) had improved the shell quality in old aged hens. In those studies, the egg production was also maintained well when the shell quality was maintained better than typical quality at those ages. But there was not such a kind of relationship in this study since the shell quality was not different among treatments. Either dietary mannanase or PKM did not influence the yolk color score in this study. It is well known that the yolk color is depended upon the characteristic of feed. In other study (Chong et al., 2008), PKM decreased the yolk color score with higher level (25%) of supplementation, but no influence with lesser level (12.5%) of supplementation. Therefore, substantially lower (5%) level of PKM supplementation in this study could be a reason why the same PKM did not induce change in yolk color score.

PKM inclusion was not beneficial for improving the most of nutrients utilization. DM, energy and total carbohydrate utilization of no PKM diets were always higher than those of PKM diets. Poor utilization of the PKM diets could be attributed to NSP, which are poor in utilization in poultry digestive system (Meng et al., 2005). However, fat utilization was always higher in PKM diet compared to no PKM diet. PKM fat contained characteristically higher proportion of C12 ~ C14 chain fatty acids (Atasie and Akinhanmi, 2009). It is also known that those fatty acids are relatively readily absorbable than other fatty acids (Ramirez et al., 2001; Ong and Goh, 2002). It is not clear why crude ash utilization was decreased by dietary mannanase supplementation. However, mannan induced changes in gut viscosity is believed to affect mineral absorption kinetics as shown in Van der Klis et al. (1995).

Humoral immune response of laying hen is known to be influenced by age of hen (Ebeid, 2011). In laying hens, yolk IgY concentration is strongly associated with transfer efficiency of serum IgG to IgY (Barua et al., 2000). Therefore, yolk IgY concentration is relatively higher in younger hens whereas serum IgG concentration is higher in older hens. This represents relatively more effective transfer metabolism in younger hens than older hens. Relatively

lower IgY concentration in PKM groups could be attributed to the quality of PKM since it varied widely in quality (Sundu et al., 2006). It is known that the poor anti-oxidative status of old aged hens could be represented as poor immune status (Ebeid, 2011). Therefore, the immune response was stimulated by anti-oxidative diet such as omega-3 fatty acid in old aged laying hen (Ebeid, 2011). The mannanase did not induce any influence on humoral immune response of old aged hen in this study, which is similar to the result of other poultry studies (Li et al., 2010; Zou et al., 2006). However, Zou et al. (2006) reported an increase of serum IgM by dietary mannanase supplementation. Therefore, the contradictory result could be attributed to either type of immunoglobulin or type of supplemental mannanase.

IMPLICATIONS

This result implied that a relatively cheaper ingredient, palm kernel meal could be successfully incorporated (5%) to the diet of old aged laying hens. In addition, β-mannanase supplementation could be beneficial to increase the feed intake of palm kernel meal incorporated diet. Further, the increased feed intake evidently exerted an increased egg production in hens aged over 73 weeks. Therefore, this study showed that the performance of old aged laying hens could be improved by both dietary PKM by stimulating feed intake and the simultaneous supplementation of β-mannanase by alleviating anti-nutritional impact of mannans in the PKM.

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