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Full Length Research

Effect of feeding ration using protein and calcium microparticles along with inulin on protein digestibility, and egg protein mass in Japanese quail

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ABSTRACT: The study aimed to assess the effect of feeding protein and calcium microparticles with the addition of *Cichorium intybus* root flour on protein digestibility and protein mass of quail eggs. 7-week-old female Japanese quails (*Coturnix coturnix japonica*) were employed, totaling 100 birds with an average body weight of 148.50 ± 3.34 g. *Cichorium intybus* root flour was utilized as a source of prebiotics. The ration consisted of corn, rice bran, soybean meal, fish meal, eggshell, premix, vegetable oil, lysine, and methionine. The study was arranged in a completely randomized design (CRD) with 4 treatments and 5 replications. The treatments applied were T0: ration with 19.15% non-microparticle protein and Ca without 0.6% chicory root flour, T1: ration with 19.10% microparticle protein and Ca without 0.6% chicory root flour, T2: T0 + 0.6% chicory root flour, and T3: T1 + 0.6% chicory root flour. Parameters measured included protein digestibility, egg fill weight, protein mass, and calcium level of quail eggs. Data were processed using analysis of variance with the F test at the 5% significance level, and if the treatment showed a significant effect, followed by the Duncan test at the 5% significance level. The results showed that feeding protein and calcium microparticles plus chicory root flour had a significant effect ($p<0.05$) on protein digestibility, egg fill weight, protein mass, and egg calcium. The conclusion is that feeding rations using protein and calcium microparticles plus chicory root flour 0.6% (T3) as a source of inulin improves protein digestibility, thereby increasing egg protein and calcium mass, but produces the same egg content weight as other treatments.

Keywords: *Coturnix coturnix japonica*, egg protein mass, microparticle, inulin, protein digestibility.

INTRODUCTION

Quail farming has a very promising opportunity to be developed because it does not require large capital and a large land area compared to other livestock. Egg production capability is high; they can produce 250-300 eggs per year with little ration consumption, about 20 g per bird per day (Akarikiya *et al.,* 2022). Egg production stability must be maintained through the provision of high-quality rations. The problem faced by all types of poultry farms is almost the same, namely, the availability of expensive protein source ration ingredients. Ration cost is the largest component of all production costs, ranging from 60 to 70% (Redoy *et al.*, 2017).

Ration costs can be reduced if the use of protein sources is reduced, but it must be anticipated that quail will not be deprived of protein intake. A possible production means is processing protein source material into micro sizes in an attempt to reduce the amount of protein in the ration without causing nutrient deficiencies in Japanese quail (Herrera *et al.,* 2018). The results of research reported by Cholis *et al.* (2018) suggested that rations with 18% and 21% microparticle protein sources produced the same carcass weight in broiler chickens. The particle size of the constituent ingredients of the protein source ration is changed to a smaller micro size through a sonification process using an ultrasound transducer. The use of rations with microparticle protein is expected to help increase livestock productivity through improved protein utilization efficiency. Previous research showed that protein digestibility increased in broilers fed fish meal and microparticle soybean meal in a single test (Suthama and Wibawa, 2018).

Protein utilization efficiency is better in rations with microparticle protein sources when additives are added. This study used a ration with microparticle protein and calcium plus chicory root meal as a source of prebiotic inulin. Both nutrients are usually transported in the form of calcium-binding protein (CaBP) to target tissues, including eggs in this study. Calcium-binding protein (CaBP) is a calcium carrier component that is formed in the intestinal mucosa and enters the blood vessels to be transported to the tissues in need (Areco *et al*., 2020). Previous research showed that protein digestibility and calcium retention increased in broilers fed rations using microparticle protein sources plus acidifier additives (Suthama *et al*., 2021). These results reinforce the assumption that CaBP formation is more adequate due to using rations with microparticle protein and calcium sources, let alone inulin.

Prebiotic inulin from chicory root is a substrate that can be selectively fermented by beneficial bacteria in the intestine but cannot be digested by the host livestock. The inulin content of chicory root (*Cichorium intybus* L.) is high, ranging from 40% to 60% (Janda *et al*., 2021). Prebiotic types of non-digestible carbohydrates, such as inulin and other oligosaccharides, serve as a source of “food” for beneficial bacteria in the digestive tract (Wilson and Whelan, 2017). Giving inulin as a feed additive can stimulate the growth of beneficial microbes, such as lactic acid bacteria (LAB), which can affect intestinal health, resulting in better digestion and absorption of nutrients (Qin *et al.,* 2023).

Therefore, the objective of this study is to assess the effect of feeding protein and calcium microparticles plus chicory root meal as a source of inulin on protein utilization and egg quality in Japanese quails.

MATERIALS AND METHODS

Livestock management

Seven-week-old 100 female Japanese quails (*Coturnix coturnix japonica*) were employed with an average body weight of 148.50 ± 3.34 g. *Cichorium intybus* was the source of inulin, which acted as a prebiotic. Rations of the treatment groups were composed of corn, rice bran, soybean meal, fish meal, eggshell, premix, vegetable oil, lysine, and methionine, with the composition and nutrient content listed in Table 1.

Disk mill type grinder were used to grind the ingredients into flour, then the ground material is sieved, and an ultrasound transducer was used to sonify the protein source ration ingredients (fish meal and soybean meal) made into microparticles. The research also uses digital scales to measure the weight of the quails, 24 battery cages for laying quails, ration and drinking containers, light bulbs, thermometers to measure the temperature and humidity of the microenvironment, and 40 and 60 mesh sieves for sieving protein and calcium sources, respectively.

Experimental procedure and design

Preparation of protein and calcium microparticles

Soybean meal as a protein source feed is pulverized first using a grinder and then sieved, but fish meal is sieved directly without going through the grinding process. The sieved soybean meal and fish meal were then sonicated with a mixture of 500 g protein source material, 1000 ml reverse osmosis (RO) water, and 10 ml virgin coconut oil (VCO). The mixture of each ration material was put into the ultrasound transducer machine for 60 minutes. The sonification results were filtered and squeezed, then dried in the sun until dry. Calcium microparticles from eggshells are made by a mechanical process that does not use chemicals. Chicken eggshells, as the source of calcium, were washed to remove the remaining egg contents and dried. The dried eggshells were then crushed with a grinder and then sieved using a 100 mesh size (150 µm) to obtain a uniform particle size.

Quail rearing, experimental design, and treatments

Quails aged 7 weeks are adapted to the environment and commercial rations until they lay at least 50% of their eggs (10 weeks old). Afterwards, quails were given a treatment ration adaptation period for 6 days, with the ratio between treatment ration and commercial ration changed periodically, namely 30:70, 50:50, 70:30, and 100:0. The treatment ration was given when the quails were 10 weeks old, as much as 20 g/head with a frequency of 4 times per day until day 60. The remaining ration was weighed every morning to measure intake. Three quail eggs for each treatment were collected and weighed for the analysis.

The study was arranged in a completely randomized design (CRD) with 4 treatments and 5 replicates. The

Table 1. Composition and nutrient content of research rations.

Feed stuff	Composition (%)			
	T0	T1	T2	T3
Yellow Corn	51.07	51.07	51.07	51.07
Rice Bran	4.60	4.60	4.60	4.60
Non-micropatellized soybean meal	24.00	0.00	24.00	0,00
Micropatellized soybean meal	0.00	24.00	0.00	24.00
Non-micropatellized fish meal	10.00	0.00	10.00	0.00
Micropatellized fish meal	0.00	10.00	0.00	10.00
Non-micropatellized egg shell	7.00	0.00	7.00	0.00
Micropatellized egg shell	0.00	7.00	0.00	7.00
Premix	2.00	2.00	2.00	2.00
Vegetable oil	1.00	1.00	1.00	1.00
Lysine	0.08	0.08	0.08	0.08
Methionine	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Nutrient content				
Metabolizable energy (kkal/kg)**	2840.78	2838.72	2840.78	2838.72
Crude protein (%)*	19.15	19.10	19.15	19.10
Crude fat (%)*	4.86	4.65	4.86	4.65
Crude fiber (%)*	4.95	4.41	4.95	4.41
Calcium (%)*	4.05	4.05	4.05	4.05
Phosphorus (%)*	0.87	0,87	0.87	0,87

Source: *Proximate and mineral analysis results at the Animal Nutrition Science Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University (2024); ** Metabolizable energy was calculated using the Bolton formula (1967).

treatments applied were T0: ration with 19.15% non-microparticle protein and Ca without 0.6% chicory root flour, T1: ration with 19.10% microparticle protein and Ca without 0.6% chicory root flour, T2: T0 + 0.6% chicory root flour, and T3: T1 + 0.6% chicory root flour.

Parameter measurement and statistical analysis

Protein digestibility was measured by the total collection method. The total collection used 100 quails for 9 days, with the addition of Fe_2O_3 as a color indicator in the treatment ratio. Excreta was collected using plastic and taken when the excreta turned red. The fresh weight of excreta was weighed and then dried in the sun and weighed again to obtain air-dried weight data. Quail eggs were broken for protein and calcium analysis by separating albumin and yolk using a syringe. Albumin and yolk were each weighed to obtain data on egg content weight, then oven-dried for 24 hours. Protein digestibility was calculated according to Krismiyanto *et al.* (2023) as follows:

$$\mathrm{PD}(\%) = \frac{\mathrm{PI - (ExP - EnP}}{\mathrm{PI}} \times 100$$

Where: PD = Protein Digestibility, PI = Protein Intake, ExP = Amount of excreta protein, and EnP = Amount of endogenous protein.

Egg protein mass was calculated by the formula according to Suthama *et al.* (2003) as follows:

Egg protein mass (g) = egg protein content × egg weight (g)

Data were analyzed using analysis of variance, applying the SPSS 16.0 statistical program. Duncan's multiple range tests examined the differences between the treatment means at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Protein digestibility

Feeding rations using protein and calcium microparticle sources with the addition of chicory root flour as a source of inulin had a significant effect ($p<0.05$) on protein digestibility in quails (Table 2). Ration using protein sources and calcium microparticles with the addition of chicory root flour (T3) produced the highest protein digestibility and was significant ($p<0.05$) compared to other treatments (T0, T1, T2). The results showed that the ratio of protein and calcium microparticles plus chicory root flour (T3) produced the highest protein digestibility (Table 2). Rations with protein sources and calcium microparticles are more easily digested and absorbed by the intestine. Previous research proved that protein sources in the form

Table 2. Protein digestibility, egg fill weight, egg protein, and calcium mass.

Treatment	T0	T1	T2	T3
Protein digestibility (%)	72,20±4,26[b]	75,33±1,44[b]	77,10±3,61[b]	83,80±2,40[a]
Egg fill weight (g)	7,52±0,53[a]	7,03± 0,52[ab]	7,23±0,67[bc]	8,39±0,70[cd]
Egg protein mass (g)	0,905±0,03[a]	0,911±0,06[b]	0,914±0,06[b]	1,602±0,08[c]
Egg calcium mass (g)	0,224±0,01[c]	0,226±0,01[bc]	0,276±0,03[abc]	0,307±0,03[ab]

Different superscripts on mean values indicate significant differences ($p<0.05$).

of fish meal and soybean meal made into microparticles and tested singly in broiler chickens increased protein and amino acid digestibility and calcium retention (Suthama and Wibawa, 2018). The effectiveness of rations using microparticle protein was enhanced by adding 0.6% inulin source from chicory root. Digestibility of nutrients, including protein and mineral calcium, increased in broilers fed diets with natural (non-microparticle) protein and calcium sources plus Lactobacillus sp. and inulin (Wu *et al*., 2019). The conditions and results achieved are very likely to be better with microparticle protein. Thus, the results of this study, especially T3, are supported by those reports, as using rations with microparticle protein and Ca sources plus chicory root flour improved protein digestibility (Table 2).

Prebiotic inulin is a source of "food" that can be fermented by beneficial bacteria in the digestive tract to produce organic acids (lactic acid) and short-chain fatty acids (SCFAs). The presence of SCFA products, even though not measured in this study, is evidenced by the inhibition of the growth of pathogenic bacteria (*coliform*) because the intestinal environment is not conducive, and conversely, the population of lactic acid bacteria (LAB) increases. The increase in LAB after being fed prebiotics in the form of inulin produces SCFAs and antimicrobial substances (bacteriocins) that can interfere with the growth of pathogenic bacteria (Krismiyanto *et al.,* 2014). A higher LAB population and lower *coliform* are indications that the digestive tract is healthier and have a positive impact on increasing nutrient digestibility, especially protein.

The non-microparticle protein ration plus chicory root meal (T2) produced protein digestibility that was not significantly different from the treatment without chicory root meal (T0 and T1). Protein digestibility of T2 was better than T0 and T1 because it was given additional chicory root flour as a source of inulin. The T2 treatment produced protein digestibility that was not as optimal as T3 because it used a non-microparticle protein ration, even though both were supplemented with chicory root inulin. Nutrient digestibility is better in broilers fed rations with regular protein (non-microparticles) plus *Lactobacillus* sp. and inulin because the bacterial balance is favourable, namely the *Lactobacillus* population is higher than the number of *E. coli* (Wu *et al.,* 2019).

Rations that were not supplemented with chicory root powder (T0 and T1) produced protein digestibility that was not different, even though T1 tended to be better. Conditions in both treatments: because of the lack of stimulus to increase the LAB population, the balance of intestinal microflora is not as good as the treatment supplemented with chicory root flour. The use of non-microparticle protein rations should be supplemented with inulin and *Lactobacillus* sp. in order to enhance the *Lactobacillus* population and suppress *E. coli* bacteria, resulting in better protein digestibility (Wu *et al.,* 2019). The use of microparticle protein rations increases the surface area of the particles and allows for more intensive enzyme accessibility, resulting in higher digestibility, but not the T0 treatment (non-microparticle protein). This result is similar to the report of Suthama and Wibawa (2018) that protein digestibility and even Ca retention increased in broilers fed fish meal and soybean meal processed with microparticles without additional additives.

Egg fill weight

Feeding rations using microparticle protein and calcium sources with the addition of chicory root flour as a source of inulin had a significant effect ($p<0.05$) on quail egg content weight (Table 2). Ration with protein and calcium microparticles with the addition of chicory root flour (T3) produced the highest egg content weight and was significantly ($p<0.05$) higher than treatments T1 and T2, but not different from T0. The weight of egg contents in the treatment using rations with microparticle protein sources without chicory root flour was significantly different from T0 and T3, but not different from T2.

Quails fed rations with protein and calcium microparticles plus chicory root flour (T3) produced the highest egg fill weights, even though not statistically different from T0 (Table 2). Factors that can affect egg weight are protein intake originating from protein digestion, associated with better and healthier digestive tract performance. Increased protein digestibility is due to the ration using microparticle protein sources and is supported by high crude fiber digestibility because important nutrients, especially protein, are not wasted with excreta. Thus, the high protein digestibility in T3 contributed to the increase in egg fill weight (Table 2).

Feeding rations using microparticle protein sources plus chicory root inulin (T3) may improve protein utilization.

Previous research showed that protein and amino acid digestibility increased in broilers fed microparticle fish meal and soybean meal protein sources (Suthama and Wibawa, 2018). This means that protein utilization can be improved by decreasing particle size. The addition of chicory root meal inulin to rations with microparticle protein completes the process to produce higher egg fill weights. This phenomenon occurs because chicory root meal inulin is a “food source” for beneficial bacteria, such as LAB, which has an indirect impact on improving nutrient digestion. The results of this study are consistent with the report of Qin *et al.* (2023) that the provision of inulin as a feed additive can stimulate the growth of LAB in the intestine, thus improving the balance of bacteria and causing better digestion and absorption of nutrients, especially protein.

Feeding rations using non-microparticle protein sources (T0) yielded significantly (p<0.05) higher egg fill weights than the treatment with microparticle protein rations (T1), however, similar to the treatment using non-microparticle protein rations plus inulin (T2). Egg fill weight is determined by differences in egg components, namely albumin and yolk. Notably, T1 was expected to be better than T0 using microparticle protein and calcium sources, but this was not the case in this study.

The addition of chicory root inulin in the T2 treatment can be stated to be of little benefit because the weight of the egg contents produced is similar to T0 (without inulin). Chickens do not show a preference for consuming ration fractions with greater protein or Ca content (Herrera *et al.,* 2018). In addition, it has been reported that chickens prefer to consume rations with coarser particle size, and this phenomenon supports the results of the study, especially T0.

The T0 treatment (non-microparticle protein only) produced the same egg fill weight as T2 (non-microparticle protein plus inulin). This implies that microparticle protein and chicory inulin are less favorable for nutrient deposition into the egg, as evidenced by both showing similar digestibility values. The same weight of yolk and albumin in both treatments (T0 and T2) is a fact that these nutrients play a role as strong contributors to the weight of egg contents (Table 2). The results of ration fat absorption are further synthesized in the liver and then carried through the bloodstream to the ovaries for yolk formation (Kaspers, 2016). Similarly, absorbed amino acids are carried to the liver and then transported to the magnum in the reproductive tract to be secreted to form albumin (Gautron *et al.*, 2023). Transportation of fat and protein in the three treatments (T0, T1, and T2) occurs through the same mechanism, as described above, as evidenced by the fact that the digestibility values of fat and protein are not different.

Egg protein mass

Feeding rations using protein and calcium microparticles with the addition of chicory root flour as a source of inulin had a significant effect (p<0.05) on quail egg protein mass (Table 2). Ration using protein and calcium microparticles with the addition of chicory root flour (T3) was significantly different (p<0.05) from all other treatments (T0, T1, T2). Egg protein mass in the treatment using non-microparticle rations without chicory root flour was not significantly different from T1 or T2.

The results of this study showed that feeding rations using protein and calcium microparticles with the addition of chicory root flour yielded the highest egg protein mass (Table 2). The value of egg protein mass is differentiated from its albumin weight because most of the egg protein content is in albumin. Abd-Elaziz *et al.* (2018) explained that egg albumin contains more than 50% of the total egg protein. High protein digestibility in T3 (Table 2) contributes to high protein availability for egg protein mass. The results of this study are supported by Purbarani *et al.* (2019), which states that inulin given to broiler chickens may improve the development of beneficial bacteria and reduce pathogenic bacteria. Improvement of bacterial balance greatly supports the health of the digestive tract to be better, and the availability of nutrients for the host increases.

Feeding rations with non-microparticle protein and calcium without the addition of chicory root flour produced the same egg protein mass as the T1 and T2 treatments and had the lowest inclination value, even though it was not tested for tendency. Protein digestibility that was not different in the three treatments (T0, T1, and T2) was also a determining factor for the same protein mass as a protein substrate for egg formation, especially albumin. Hou *et al.* (2020) explain that ration protein is hydrolyzed in the digestive process into amino acids, then absorbed by intestinal cells, and then transported to the liver. Amino acids in the liver become yolk precursors, and some are taken to the magnum for egg white formation. The above mechanism occurs in all three treatments (T0, T1, and T2), but at a very mild level; otherwise, the process is most effective when the ratio is with protein and calcium microparticles plus chicory root inulin (T3). The effect of using protein microparticles, also with the addition of inulin, did not increase protein digestibility. This is different from what was reported by Suthama and Wibawa (2018) and Suthama *et al*. (2021), that feeding microparticle protein to broilers increases protein and amino acid digestibility. The case in this study, even though fed a ration with protein and calcium microparticles or the addition of inulin, did not affect protein digestibility and yielded the same egg protein mass. It can be assumed that two things are true: first, protein intake is more repositioned for meat; second, there are differences in response between quail and broiler.

Egg calcium mass

Feeding rations using protein and calcium microparticles with the addition of chicory root flour as a source of inulin had a significant effect ($p<0.05$) on the calcium mass of quail eggs (Table 2). Feeding rations using both non-microparticle and microparticle protein and calcium with added chicory root meal (T2 and T3) yielded significantly ($p<0.05$) higher calcium mass than the other two treatments (T0 and T1). The two treatments (T2 and T3) did not differ, nor did T0 and T1.

Feeding rations with protein and calcium microparticles plus chicory root meal (T3) produced the highest egg calcium mass, even though statistically not different from T2 (Table 2). The high mass of egg calcium is related to the availability of calcium or calcium that can be absorbed. High protein digestibility in the T3 treatment (Table 2) affects calcium availability because calcium is usually absorbed and always binds to protein in the form of calcium-binding protein (CaBP). Calcium retained or absorbed increases with high protein digestibility because calcium is metabolized with a protein called CaBP (Hou *et al.,* 2020). If CaBP formation increases, it is possible that more calcium can be transported into target tissues, especially eggs, as quails are in their laying period. Similarly, Areco *et al.* (2020) stated that CaBP is a calcium carrier component that is formed in the intestinal mucosa and enters the blood vessels to be transported to the tissues in need. The mechanism mentioned above is very influential on the process of calcium metabolism, including in eggs in this study.

The above phenomenon occurs in the T2 treatment, which is related to changes in the condition of the digestive tract due to the addition of chicory root as a source of inulin. Feeding rations using both microparticle and non-microparticle protein and calcium, with the addition of chicory root flour (T2) and (T3) produced significantly higher egg calcium mass than the other two treatments (Table 2). Egg calcium mass in treatment T3 tended to be higher than in T2, even though no trend was tested, due to the superiority of calcium metabolic processes as discussed earlier. Egg calcium mass is closely related to the calcium retention value. The higher the calcium retention value, the greater the amount absorbed, thereby enabling the calcium mass of eggs to increase. Effective calcium absorption occurs under low intestinal pH conditions. Acidic intestinal conditions are likely due to the fermentation process of inulin by LAB in both treatments, which can produce SCFA. The condition in this study is reinforced by Fernandez *et al.* (2016) report that LAB produces SCFAs, including acetate, propionate, and butyrate, through prebiotic fermentation, which can reduce intestinal pH.

Feeding rations with different forms of protein sources, non-microparticles (T0) and microparticles (T1), both without the addition of chicory root flour, yielded no different egg calcium mass. The egg calcium mass of T1 is expected to be better than T0 because it uses a microparticle protein source. Similar values and low values of egg calcium mass in both treatments were influenced by available calcium and provider factors. Protein digestibility (Table 2) in treatments T0 and T1 showed similarly low values due to inadequate contribution of protein and calcium binding in the form of CaBP to egg calcium mass. Protein digestibility and calcium retention increased in broilers fed diets using microparticle protein sources (Suthama *et al.,* 2021), as discussed earlier. This phenomenon did not occur in the T1 treatment due to differences in response between broiler and quail, resulting in egg calcium mass similar to T0 (Table 2).

Conclusion

Feeding rations using protein and calcium microparticles plus chicory root flour 0.6% (T3) as a source of inulin improves protein digestibility, thereby increasing egg protein and calcium mass, but produces the same egg content weight as other treatments.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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