Original Article

Effects of Conditioning Methods on the Physical and Microbiological Quality of Broiler Pelleted Feeds

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ABSTRACT

Background: The physical and hygienic quality of pelleted feed can be affected by various factors, including temperature and retention time during the conditioning process.

Objectives: This study aimed to compare the effects of producing pellets with conventional and super-conditioner methods at various temperatures and retention times on pellet quality in term of microbial and physical parameters and the recovery rate of phytase.

Methods: Eight broiler diets were prepared, including mash feed, conventional pellets (COP), and 6 super-conditioner pellets (SUP), processed at 70 ˚C, 80 ˚C, and 90 ˚C for 3 and 6 minutes. The total counts of aerobic bacteria and fungi, the pellet durability index (PDI), hardness, and the recovery rate of the included phytase were then compared among the treatments.

Results: Compared to the mash feed, the total number of viable bacteria in the COP and SUP treatments decreased as the temperature and retention times increased. The fungal population was significantly lower in the pellet treatments than in the mash feed; furthermore, the SUP treatments exhibited a lower fungal count than the COP treatment. The PDI were higher ($P \leq 0.05$) in the SUP80 and SUP90 treatments than in the COP and SUP70 treatments. Pellet hardness was also higher ($P \leq 0.05$) in the SUP90 treatments than the other pellet treatments. However, phytase activity in the pelleted feeds significantly decreased compared with the mash feed.

Conclusion: The results indicate that an increase in the conditioner temperature may improve feed quality parameters; a conditioner temperature of 80 ˚C is recommended for optimal broiler chicken pellet production when using the super-conditioner method.

Keywords: Hardness, Pelleted feed, Pellet durability index, Phytase, Super conditioner

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Introduction

Feed constitutes 60-70% of the expenses in the broiler rearing industry, and the quality of the feed plays a significant role in the flock's performance. Feed processing accounts for 2-3% of the total feed production costs and includes various processes, such as grinding, mixing, pelleting, expanding, and extruding (Boroojeni et al., 2016). Pelleted feed is the most common form of feed in broiler rearing (Abdollahi et al., 2013b). Benefits of pelleted feed in broilers include increased feed consumption, weight gain, improved feed conversion rates, reduced feed wastage, better flock uniformity, elimination of feed selectivity, increased feed density, easier feed transportation, and improved feed palatability and hygiene (Abdollahi et al., 2019; Abdollahi et al., 2013a; Idan et al., 2020). The temperature and pressure during the process of pellet production lead to starch gelatinization, protein denaturation, the breakdown of anti-nutritional substances, and enhanced starch and protein absorption (Abdollahi et al., 2013b).

It can also help eliminate pathogens, especially *Salmonella* spp. and phathogenic coliform bacteria when processed at temperatures above 80 ˚C. Spore-forming bacteria, however, may survive at lower temperatures, below 110 ˚C (Kiarie & Mills, 2019; Boroojeni et al., 2016). However, the thermal process involved in pellet production can have a detrimental effect on supplements and additives, such as vitamins, amino acids, and enzymes (Abdollahi et al., 2013b).

The main advantages of pelleted feed over mash feed are related to the physical quality of the pellets (Idan et al., 2020; Massuquetto et al., 2020). Pellet quality is influenced by several factors, including formulation (40%), particle size (20%), conditioner temperature and duration (20%), die (15%), and dryer and cooler (5%). When formulating feed, nutritionists prioritize factors, such as meeting nutritional needs, ingredient costs, and availability over physical pellet quality. In a study conducted by Rueda et al., it was found that the temperature and moisture of the feed in the conditioner increased with the injection of steam. This increase in temperature and moisture contributed to improved starch gelatinization and enhanced pellet physical quality (Rueda et al., 2022). The moisture content of the feed increases with a longer retention time or higher temperature through steam injection in the conditioner. This, in turn, enhances starch gelatinization. However, the increased moisture also reduces friction between the feed and the die wall due to lubrication, which can lead to reduced starch gelatinization within the die. Achieving the desired physical quality of the pellet relies on finding the optimal balance between temperature, feed retention time in the conditioner, and die wall friction (Abdollahi et al., 2013b).

Myo-inositol hexaphosphate (phytate) is the primary storage form of phosphorus in cereals and legumes, often combined with amino acids and minerals, such as potassium, magnesium, and calcium. Due to the absence of endogenous enzymes in monogastric animals, phytate has low digestibility and is excreted in the feces. Phytase breaks down the phytate, improving the digestibility of phosphorus, minerals, and amino acids (Selle et al., 2023; Truelock et al., 2022). For the enzyme to be effective, it must remain active when it reaches the digestive system. Maintaining the activity of the phytase enzyme during pellet production is crucial, as temperature and pressure can negatively affect its function (Truelock et al., 2022). Several solutions have been proposed to protect the phytase enzyme from the hydrothermal process, including enzyme coating, developing more heat-resistant enzymes through genetic engineering, and spraying a liquid enzyme after pellet production. However, these options are often costly or require specialized equipment (Slominski et al., 2007).

By adding micronutrients and additives after the heating process, the super-conditioner device (SUP) helps prevent the destruction of these materials. This method also allows the produced pellets to undergo additional heating processes compared to conventional methods. An advantage of the SUP is that it includes a dryer after conditioning the feed mixture (Soltan et al., 2020; Malekkhahi et al., 2021) which helps remove excess moisture from the feed and eliminate the lubricating effect of moisture caused by the conditioner. The objective of this experiment was to study the effects of conditioner temperatures and retention times on pellet quality parameters and the recovery rate of phytase enzyme, using SUP methods in comparison with conventional pellet (COP) production methods.

Materials and Methods

Treatment diets

The starter corn-soy diet was formulated (Table 1) according to the standard recommended for broiler chickens (Aviagen, 2018). The phytase enzyme used in this research was a heat-resistant 6-phytase enzyme produced by *Escherichia coli* bacteria (Endophos, South Korea). The enzyme was added at a rate of 50 grams per ton to the feed (0.5 IU/g) during the specified stage in each

Table 1. Composition of broiler starter diets

Ingredient	% as-fed
Corn	48.99
Wheat	8
Soybean meal, 44% crude protein	38.26
Soybean oil	0.56
Calcium carbonate	1.2
Di-calcium phosphate	1.74
Salt	0.3
DL-methionine	0.26
L-lysine HCl	0.19
Trace mineral premix	0.25
Vitamin premix	0.25
Total	100

treatment. The study was designed with eight treatments. The ingredients were first ground using a hammer mill (Asiab Company, Tehran, Iran). For the production of mash feed, all ingredients were added to a mixer (Asiab Company, Tehran, Iran) and blended with other micronutrients and additives. To provide the COP first, the ingredients were mixed as per the mash feed treatment and then conditioned at 75 ˚C for 30 seconds. The mixture was then processed through a die with a hole diameter of 1.8 mm and a thickness of 45 mm (Salmatek, Germany) to produce the pellets.

The SUP treatments included SUP 70 ˚C - 3 minutes, SUP 70 ˚C - 6 minutes, SUP 80 ˚C- 3 minutes, SUP 80 ˚C- 6 minutes, SUP 90 ˚C - 3 minutes, and SUP 90 ˚C - 6 minutes, all produced using the SUP method. For these treatments, the mixed ingredients were fed into the super-conditioner, where they underwent a hydrothermal process at temperatures of 70 ˚C, 80 ˚C, and 90 ˚C each for 3 and 6 minutes. Subsequently, the conditioned feed was dried in a dryer, and an additive was incorporated and mixed. Finally, the SUP treatments were completed by conditioning the mixture and passing it through the die. After producing the treatments, three samples from each were taken, stored in plastic bags at 4 ˚C until the tests were performed.

Microbial testing was conducted at the laboratory of the Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran.

Enumeration of microbial population:

Total viable count and total fungal colony count

To determine the total number of aerobic bacteria and the total viable count (TVC), 90 mL of a 0.9% sodium chloride solution was added to 10 g of the ground sample in a sterile Falcon tube and shaken. Serial dilutions were then prepared from the resulting solution, ranging from 10^{-1} to 10^{-5}. From each dilution, 100 µL was cultured in triplicate using nutrient agar medium (Ibresco, Iran) and Sabouraud dextrose agar medium containing the antibiotic chloramphenicol (Merck, Germany) for TVC and total fungal colony count (TFC), respectively.

All cultures were maintained at room temperature for 10 minutes before incubation. The nutrient agar medium was incubated at 37 ˚C for 24 hours, while the Sabouraud dextrose agar medium was incubated at 28 ˚C for 72 hours. After the incubation period, bacterial and fungal colonies were counted in the culturea that had between 30 and 300 colonies (Arotupin et al., 2007).

Coliform bacteria count

The most probable number (MPN) method was employed to count coliform bacteria. Ninety milliliters of peptone broth medium were added to 10 g of the homogenized samples and incubated at 37 ˚C for 24 hours. Then, 10 mL of the samples were cultured in three tubes

containing double-strength lactose broth, each with a Durham tube; 1 mL of the sample was then cultured in three lactose broth tubes, also with a Durham tube. Next, 0.1 mL of the sample was cultured in three lactose broth tubes, each with a Durham tube. The medium was then incubated at 37 ˚C for 24 hours. Samples from the positive gas tubes were cultured in a Brilliant Green medium with a Durham tube and then incubated. The number of positive gas tubes was used to estimate the number of coliform bacteria present. To confirm *E. coli* from other gas-positive coliform bacteria, suspicious samples were cultured in MacConkey medium and incubated at 37 ˚C for 24 hours. Biochemical tests, including TSIA (Triple Sugar Iron Agar), Motility, MR (Methyl Red), Indole, H2S, Citrate, and Urease, were also performed on the red colonies to confirm the diagnosis (Arotupin et al., 2007).

Pellet durability index (PDI) measurement

To determine the PDI, 500 g of the samples were sieved using a 1.4 mm mesh sieve to separate the powder from the pellets. The whole pellets were then weighed and placed into a tumbling can machine (Faculty of Agricultural Technology, Aburihan Campus, University of Tehran, Tehran, Iran). The pellets were shaken for 10 minutes at 50 rpm and then weighed again. After sieving, the PDI percentage was calculated using the Equation 1 (Abadi et al., 2019; Evans et al., 2021):

1. *PDI (%)=(Pellet weight after tumbling / Pellet weight before tumbling)×100*

Hardness measurement

To measure hardness, 20 pellets from each treatments were selected and evaluated using a hardness tester (Faculty of Agricultural Technology Aburihan Campus, University of Tehran, Tehran, Iran). The average force required was recorded in kilograms of force (kgf).

Measurement of phytase activity

A colorimetric method was employed to measure phytase activity. Treatment samples were ground and mixed. Then, 2.5 g of each sample was placed in an Erlenmeyer flask, and 20 mL of 0.2 M sodium acetate buffer solution (pH 5) was added. The mixture was shaken for 30 minutes at room temperature. The contents of the flask were then centrifuged for 10 minutes at 2000 rpm. Then, 1 mL of the supernatant was transferred into a tube and the tube was placed in a bain-marie at 37 ˚C for 5 minutes. Subsequently, 1 mL of substrate was added to the tube, which was then mixed and incubated in a bain-marie at 37 ˚C for 15 minutes. The reaction was stopped by adding 2 mL of stop solution to all tubes. A blank tube was prepared by adding the stop solution before the substrate.

Next, 0.15 mL from both the blank and sample tubes were transferred to new tubes, and 1.35 mL of sterile water was added to each tube. Then, 1.5 mL of reagent C solution (3 volumes of 1 M sulphuric acid with 1 volume of 2.5 % ammonium molybdate, followed by adding 1 volume of 10% ascorbic acid, and mixing well) was added to the tubes. The tubes were incubated in a bain-marie at 50 ˚C for 20 minutes. After incubation, the tubes were centrifuged, and the absorbance of each tube was measured against distilled water at a wavelength of 820 nm.

To prepare standard solutions, 612.4 mg of KH_2PO_4 was dissolved in 500 mL of water to create a phosphate stock solution with a concentration of 9 mM. From this stock solution, dilutions of 1/100, 1/200, and 1/400 were made, resulting in phosphorus concentrations of 90, 45, and 22.5 nmol/mL, respectively. The phosphorus content in these standard solutions was then measured and phytase activity of the samples was determined using the standard curve. One unit of phytase activity was defined as the amount of enzyme that liberates 1 µmol of inorganic phosphate from sodium phytate in 1 minute under assay conditions. The percentage recovery rate (PRR) of phytase was calculated using the Equation 2 (Kim & Lei, 2005):

2. *PRR=(PB/PA)×100*

Where, PB is the amount of phytase enzyme in feed after pelleting and PA is the amount of phytase enzyme in the feed before pelleting.

Statistical analysis

The results for microbial population, physical quality of pellet samples, and enzyme activity were analyzed using Minitab statistical software (version 18) and the analysis of variance (ANOVA). Differences between means were compared using the Tukey's test, with significance assessed at the $P<0.05$ level.

Results

Microbial populations

The results of the microbial enumeration are presented in Table 2. The total number of aerobic bacteria and fungi in the mash diet was 9.4×10^7 CFU/g and 3.5×10^2 CFU/g, respectively. For the COP, these values were 5.95×10^7 CFU/g and 2.18×10^2 CFU/g, respectively. The total via-

Table 2. Effect of conditioner temperature and retention time on microbial population of mash and pelleted diets produced by conventional and super-conditioner methods

Treatments	TVC (×10^5 CFU/g)*	TFC (×10^2 CFU/g)	TCC
Mash	940±19	3.5±0.1^a	700±200
COP	595±45	2.18±0.17^b	ND
SUP70-3	567.7±87.78	1.9±0.3^c	ND
SUP70-6	146.6±21.82	2.±2.6^c	ND
SUP80-3	123.3±27.2	1.5±0.3^d	ND
SUP80-6	52.7±31.5	1±0^d	ND
SUP90-3	11.4±7.37	1.2±0.2^d	ND
SUP90-6	12.4±2.8	1.03±0.12^d	ND
P		0.0001	

Abbreviations: TVC: Total viable bacterial count, TFC: Total fungal colony-forming unit, TCC: Total coliform count, COP: Conventional pellet, SUP70-3: Super-conditioner pellet at 70 ˚C for 3 minutes, SUP70-6: Super-conditioner pellet at 70 ˚C for 6 minutes, SUP80-3: Super-conditioner pellet at 80 ˚C for 3 minutes, SUP80-6: Super-conditioner pellet at 80 ˚C for 6 minutes, SUP90-3: Super-conditioner pellet at 90 ˚C for 3 minutes, SUP90-6: Super-conditioner pellet 90 ˚C for 6 minutes; ND: Not detected.

*Due to the non-normal distribution of the data, statistical comparisons were not possible.

Note: Means within the same column with different superscript letters are significantly different (P<0.05).

ble bacteria in the SUP treatments ranged from 1.24×10^6 to 5.67×10^7 CFU/g, with the highest count observed in the treatment SUP70-3 and the lowest in the SUP90-6 treatment. The total fungal colony-forming units in the SUP treatments varied from 100 to 200 CFU/g in which the highest number was related to the SUP70-6 treatment and the lowest number was associated with the SUP80-6 treatment.

The pelleting process resulted in a significant (P<0.001) reduction in the fungal population, which a more pronounced decrease observed in the SUP treatments compared to that of the COP treatment. Notably, in the SUP treatments, increasing the temperature from 70 ˚C to 90 ˚C caused a significant (P<0.05) drop in fungal counts. However, at temperatures of 80 ˚C and 90 ˚C, the difference in fungal colony counts between the treatments was not significant. Regarding coliform bacteria, only the mash feed was contaminated, with a count of 700 CFU/g. No coliform bacteria and *E. coli* were detected in the other treatment cultures.

Pellet quality parameters

The results for PDI and hardness are presented in Table 3. The PDI for the COP was 92.53%, while for the SUP treatments, the values at temperatures of 70 ˚C, 80 ˚C, and 90 ˚C were 94.37%, 96.39%, and 97.75%, respectively. As the conditioner temperature increased, the PDI increased significantly (P<0.001). The PDI of pellets produced by the SUP method at both 80 ˚C and 90 ˚C was significantly (P<0.001) higher than that of the COP. A linear relationship between conditioner temperature and the PDI was also observed across the test treatments (r^2=0.987). A significant difference (P<0.007) was also observed between the PDI of COP and SUP treatments due to the difference in conditioner retention time. The values were 92.53%, 95.96%, and 96.38% at 30 seconds, 3, and 6 minutes, respectively, but no significant difference was observed among the SUP treatments.

The hardness of the COP treatment was 7.67 kgf, while the mean values for the SUP treatments at 70, 80, and 90 ˚C were 8.62, 9.97, and 13.17 kgf, respectively. No significant difference in hardness was observed between the COP and the SUP70 ˚C and SUP80-3 treatments however, significant (P<0.001) differences were observed between the SUP80-6, SUP90-3, and SUP90-6 treatments. Additionally, a strong positive linear relationship (r^2=0.948) was observed between temperature and the hardness index in the SUP treatments.

Phytase activity

The results of phytase activity are shown in Table 3. Phytase activity in the mash feed was 0.629 IU/g, which was significantly higher than in all other treatments (P<0.001). The enzyme activity in the COP (0.559 IU/g) was also significantly (P<0.001) higher than that of the SUP treatments, except for SUP70-3. In the SUP treatments, phytase activity ranged from 0.477 to 0.496 IU/g, and no significant differences were observed between the treatments. The enzyme recovery rate in the COP was 88.87%, while it was lower (75.83% to 83.78%) for the pellets produced by the SUP method. Furthermore, the effect of conditioner temperature and feed retention time on phytase activity in the SUP treatments was not significant.

Discussion

Feed contamination with pathogenic agents presents a potential risk in broiler production, as it can lead to disease outbreaks and reduced flock performance. Additionally, there is a risk of transmitting these pathogens to humans through the consumption of broiler products. In this study, the hydrothermal process in all treatments resulted in a decrease of less than one Log10 in the total fungi. The TVC showed also a decrease in the treatments due to the hydrothermal process (Table 2). The TVC and TFC showed a reduction by increasing both the temperature and duration of the hydrothermal process. The mean reduction in the microbial load when the temperature increased from 70 ˚C to 80 ˚C and from 80 ˚C to 90 ˚C was about one-forth in the SUP treatments. Spore-forming bacteria, which are resistant to temperatures up to 110 ˚C, were not destroyed by the heat and pelleting process (Boroojeni et al., 2016). An increase in conditioner temperature may be associated with a decrease in non-spore-forming and active bacteria. At temperatures above 80 ˚C, most of the bacterial population in the pellet feed is comprised of spore-forming bacteria (Boroojeni et al., 2016). Similar results were observed in a study by Cox et al. (1986). Furuta et al. (1980) investigated the impact of conditioner temperature on TVC and found that increasing the conditioner temperature from 70 ˚C to 90 ˚C reduced the microbial load by 3 Log10. This contrasts with

Table 3. Effect of conditioner temperature and retention time on PDI, hardness, and phytase activity of pelleted diet produced by super-conditioner and conventional methods

Treatments	PDI (%)	Hardness (Kgf)	Phytase Activity (IU/g)	Phytase Recovery Rate (%)
Mash	-	-	0.629^{a}	100
COP	92.53^{a}	7.67^{a}	0.559^{b}	88.87
SUP70-3 min	93.92^{ac}	8.30^{ac}	0.527^{bc}	83.78
SUP70-6 min	94.82^{acd}	8.94^{ac}	0.494^{cd}	78.53
SUP80-3 min	96.01^{bc}	9.66^{ac}	0.496^{cd}	78.85
SUP80-6 min	96.78^{bd}	10.29^{bc}	0.477^{d}	75.83
SUP90-3 min	97.95^{b}	12.96^{d}	0.496^{d}	78.85
SUP90-6 min	97.56^{b}	13.38^{d}	0.484^{cd}	76.94
SEM	0.342	0.4388	0.005	
P				
Treatments	<0.001	<0.001	0.0001	-
Temperature effects	<0.001	<0.001	0.092	-
Time effects	>0.05	>0.05	0.177	-

Abbreviations: PDI: Pellet durability index; COP: Conventional pellet; SUP70-3: Super-conditioner pellet at 70 ˚C for 3 minutes; SUP70-6: Super-conditioner pellet at 70 ˚C for 6 minutes; SUP80-3: Super-conditioner pellet at 80 ˚C for 3 minutes; SUP80-6: super-conditioner pellet at 80 ˚C for 6 minutes; SUP90-3: Super-conditioner pellet at 90 ˚C for 3 minutes; SUP90-6: Super-conditioner pellet 90 ˚C for 6 minutes.

Note: Means within the same column with different superscript letters are significantly different (P<0.05).

the results of the current study, and the difference may be due to variations in the bacterial flora in the diet and the remaining spore-forming bacteria population after the hydrothermal process (Boroojeni et al., 2016).

Coliform bacteria, particularly *E. coli*, serve as indicators of feed contamination with feces and highlight potential routes for transmitting pathogenic agents from the feed (Maciorowski et al., 2007). In the mash feed, the *E. coli* count was 7.0×10^2 CFU/g, while no coliform bacteria were detected in the other treatments. Similar studies by Furuta et al. (1980), Cox et al. (1986), and Boltz et al. (2019) found that all pellet treatments were negative for coliform bacteria after hydrothermal processing, which aligns with the findings of the current study.

The hygienic condition of the pellets can be influenced by four factors in the conditioner, including temperature, retention time, humidity, and pressure (Boroojeni et al., 2016). Steam pressure in conditioners typically ranges from 138 to 552 kPa. When steam is injected into the conditioner, the moisture content of the feed increases, along with the retention time. As the temperature rises, more steam is introduced, resulting in a higher feed humidity. Additionally, extending the retention time allows the steam to penetrate the feed more effectively (Boroojeni et al., 2016). Conditioning the feed at temperatures above 80 ˚C and pressures below 392.26 kPa, with 15% humidity for 10 seconds, effectively eliminates coliform bacteria and reduces the overall bacterial count in the feed (Boroojeni et al., 2016). Based on the results of this research, hydrothermal processing at 70 ˚C for 3 minutes or 75 ˚C for 30 seconds can effectively eliminate coliform bacteria.

In this study, raising the conditioner temperature in the SUP treatments significantly ($P<0.001$) improved the PDI in the SUP80 and SUP90 treatments compared to that of SUP70. A strong positive linear relationship ($r^2=0.987$) was also observed between temperature and PDI in the SUP treatments. Various studies have reported similar findings on the effect of increasing conditioner temperature to improve PDI (dos Santos et al., 2020; Netto et al., 2019; Rueda et al., 2022). Netto et al. reported that the PDI of starter feed produced with a 4.7 mm die increased linearly from 78.8% to 91.4% ($r^2=0.94$) when the conditioner temperature was raised from 50 ˚C to 90 ˚C (Netto et al. 2019). This result is consistent with the findings of the current study. The difference in PDI between this study and Netto et al.'s study may be attributed to variations in die diameter, extended conditioning time in the SUP method, the removal of excess moisture in the dryer, and the elimination of the lubricating effect of moisture on the die. If steam injection is used to achieve higher temperatures or extend the feed retention time in the conditioner, feed moisture may increase. If moisture exceeds 18%, the feed becomes pasty, which can cause the pelletizing machine to stop working (Čolović et al., 2010). However, in the super-conditioner device, the reduction of humidity after the super-conditioner stage prevents this issue.

Rueda et al. (2022) reported that raising the conditioner temperature from 71 ˚C to 88 ˚C led to an increase in the PDI for both grower and finisher pellets. The PDI increased from 88.42% to 98.15% and from 86.95% to 94.61% for grower and finisher pellets, respectively. These findings are consistent with the results of our study, even though a larger die (4 mm) was used by them.

Netto et al. (2019) reported that increasing the conditioner temperature from 50 ˚C to 90 ˚C resulted in an increase in force from 4.32 to 7.53 kgf. These findings are consistent with the results observed in the current study in the SUP treatments (8.3 to 13.38). However, in this study, the hardness value was significantly higher than their findings. This discrepancy may be attributed to the impact of the super-conditioner or variations in the diameter of the die-holes. It has also demonstrated that increasing the conditioner temperature enhanced the hardness index, which aligns with the findings of the present study (dos Santos et al. 2020).

The retention time of the feed in the conditioner is another variable that can significantly affect pellet quality (dos Santos et al., 2020). In our study, we observed a significant ($P<0.001$) difference in the PDI and hardness between pellets conditioned for 30 seconds (COP) and those conditioned for 3 and 6 minutes (SUP) at both 80 and 90 ˚C. However, no substantial difference was found between the 3- and 6-minute conditioning times in these parameters.

In a study conducted by dos Santos et al. (2020), extending the feed retention time in the conditioner from 3 to 20 seconds at temperatures of 65 ˚C and 85 ˚C led to significant improvements in both the PDI and hardness. Similarly, in a study by Massuquetto et al. (2018), where the conditioner retention time ranged from 0 to 120 seconds and the temperature was maintained at 58.7 ˚C across all treatments, it was found that conditioning the pellets for 60 seconds improved the PDI by 5.9% compared to pellets treated without conditioning. However, no significant difference was observed in PDI for pellets conditioned for longer periods (60, 80, 100, and 120 seconds) (dos Santos et al., 2020; Massuquetto et al., 2018).

The effect of retention time on pellet hardness was significant (P<0.01) in the SUP treatments compared to COP, but no such effect was observed between the SUP treatments. This finding aligns with the research of dos Santos et al. (2020) and Massuquetto et al. (2018), who reported that increasing conditioner time from 3 to 60 seconds significantly improved the physical quality of the pellets. However, when conditioning time exceeded 60 seconds, this effect diminished.

One of the primary concerns regarding the use of phytase enzymes in broiler feed is the detrimental impact of the thermal process during pellet production on the enzyme's bioavailability. In this study, the enzyme recovery rate was 88.87% in the COP. Evans et al. (2021) found that the enzyme recovery rate at 74 ˚C with a 30-second retention time ranged from 86.6% to 90.9%. It is important to note that we used a heat-resistant enzyme derived from *E. coli*, while they used a heat-resistant fungal enzyme from *Trichoderma reesei*. In another study (Truelock et al. 2019), the enzyme recovery ranged from 72.24% to 78.1% when a heat-resistant enzyme was used at a conditioner temperature of 74 ˚C for a 30-second retention time (HiPhos 2700), which are consistent with the present results. In this study, no significant relationship was found between the temperature retention time of the conditioner on the recovery rate of phytase in the SUP70-6, SUP80, and SUP90 treatments. This findings highlights the ability of the super-conditioner device to protect the phytase enzyme from the thermal process up to 90 ˚C.

Despite the lack of a significant effect between the SUP treatments on phytase activity, the observed numerical decrease in phytase activity may associated with higher conditioner temperatures due to the longer cooling time required for the feed after passing through the SUP. In a study by Pope and Fahrenholz (2020), raising the conditioner temperature from 80 ˚C to 92 ˚C resulted in a significant decrease in phytase enzyme from 77.1% to 5.2%. This contrasts with the results of the current study, where the enzyme's exposure to high temperatures during conditioning did not lead to such a dramatic reduction in activity.

In this study, the highest level of phytase activity was found in the mash feed, which underwent no thermal processing. Among the pellet treatments, the recovery rate of the phytase was greater in the COP compared to the SUP treatments. Several studies have shown that when the conditioner temperature is below 74 ˚C, the friction between the feed and the die wall plays a significant role in inactivating the phytase enzyme. However, at higher conditioner temperatures (80-90 ˚C), the conditioner itself becomes more critical in the inactivation of phytase enzyme.

In this research, for the COP and SUP70-3 and SUP70-6 treatments, due to the lower conditioner temperatures, the primary reduction in enzyme activity may attributed to the die and probably to the inactivation of the endogenous enzyme. In both the SUP80 and SUP90 treatments, where no enzyme was added during the thermal process, only the inactivation of the endogenous enzyme may have occurred. Additionally, because feed moisture was removed before the phytase enzyme was added, the pattern of phytase enzyme inactivation mirrored that of the COP, highlighting the die's greater role in inactivating the phytase enzyme.

Conclusion

The results indicate that the thermal process may positively impact pellet hygienic quality in both the CON and SUP production methods. As the conditioner temperature increases in the SUP, both the PDI and hardness of the resulting pellet increase linearly, demonstrating the significant role of conditioner temperature in enhancing the physical quality of the pellets. The pelleting process significantly (P<0.001) reduces phytase activity compared to the mash treatment. However, pellet treatments produced using the SUP method can effectively maintain enzyme activity at higher temperatures and for longer retention times, without compromising their effectiveness. Overall, a conditioner temperature of 80 ˚C with a 6-minute retention time in the SUP method could be an optimal condition for producing high-quality pellets for broiler chickens.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization, formal analysis, and supervision: Mohammad Rezaeian; Data curation, and writing the original draft: Mohammad Sadegh Moradi; Review and editing: Ahmad Madani Seyed and Mohammad Rezaeia; Methodology, investigation, and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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