Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2014 / v.16 / n.4 / 367-374

http://dx.doi.org/10.1590/1516-635x1604367-374

Impact of Particle Size, Thermal Processing, Fat Inclusion, and Moisture Addition on Starch Gelatinization of Broiler Feeds

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Keywords

Conditioning, expander, gelatinization, moisture, starch, fat.

Abbreviations: PDI, pellet durability index; TP, thermal processing; FI, fat inclusion; MA, moisture addition; PS, particle size.

Submitted: August/2013
Approved: April/2014

ABSTRACT

The present study evaluated the effect of feed particle size, thermal processing different levels of fat inclusion and of moisture addition on the amount of gelatinized starch in a corn-soybean broiler diet. The different processing factors were combined in a 2 x 4 x 4 x 2 factorial arrangement in a three randomized block design consisting of three production series: two particle sizes (coarse: 1041 microns and medium: 743 microns), four fat inclusion levels at the mixer (15, 25, 35, and 45 g/kg of feed), four moisture addition levels in the conditioner (0, 7, 14, and 21g/kg of feed), and two thermal processing treatments (conditioning-pelleting or conditioning-expanding-pelleting) which resulted in 64 different processed feeds. For the determination of the amount of gelatinized starch one feed sample was collected per treatment in each of three production series, totaling three replicates/treatment. Data were transformed using a variation of Box-Cox transformation in order to fit normal distribution ($p>0.05$). Adding moisture up to 21g/kg of feed in the conditioner linearly increased the amount of gelatinized starch ($p<0.05$). The conditioner-expander-pelleting treatment of the diets (at 110°C) increased ($p<0.05$) the degree of starch gelatinization from 32.0 to 35.3 % compared with the conditioner-pelleting treatment (at 80-82°C). The gelatinized starch content increased from 30.2 to 37.2% in the feed ($p<0.05$) as the particle size increased from medium to coarse. Fat inclusion had a quadratic effect ($p<0.05$) on starch gelatinization. The degree of starch gelatinization was significantly reduced with fat inclusion levels higher than 35 g/kg of diet. The factors evaluated in this study resulted in interactions and significant effects on degree of starch gelatinization.

INTRODUCTION

Starch is stored in plants as granules. The starch granule is a structure composed by crystalline and amorphous regions and, due to the lower order of cristallinity, the gelatinization occurs first in the amorphous region than in the crystalline region (Lund & Lorenz, 1984). When the starch granule is submitted to heat, moisture, and shear force during feed expansion and or conditioning a phenomenon known as starch gelatinization may occur. Gelatinization is an irreversible process: water diffuses into the starch granule, hydrogen bonds are disrupted, the granule swells and loses shape, and amylose begins to leach (Holm *et al.*, 1988; Lund & Lorenz, 1984; Moritz *et al.*, 2005; Coral *et al.*, 2009). White *et al.* (2008) reported that the changes in the optical properties of starch can be monitored by the disappearance of 'Maltese crosses' and the disappearance of A-type diffraction patterns in the X-ray diffractograms when wheat granules are submitted to extrusion. The temperature at which starch loses its crystallinity and the material diffuses from the granules is called the "gelatinization temperature". Accordingly to Lund & Lorenz (1984), for a population of granules, the gelatinization temperature varies between 5 to 10°C, and therefore, different starch granules in a sample submitted to a heat treatment may exhibit different gelatinization degrees.

Many factors present in a feed mill can affect starch gelatinization. Moritz *et al.* (2005) and Tran *et al.* (2007) observed that different processing conditions (higher temperature and shear forces) can affect starch gelatinization rate. When these authors steam-conditioned corn at 65.6°C for 10 seconds, followed by further pelleting, they observed that gelatinized starch percentage increased from 0% to 28.58%; on the other hand, when the same corn was submitted to extrusion (barrel chamber temperature set at 48, 48, 90, 120, 150, 142, 142 and 152°C), gelatinized starch percentage reached 91.52%.

Moritz *et al.* (2001) observed that starch gelatinization increased due to added moisture; they reported that increasing the moisture content of mash feed from 6.97 to 14.47% prior to conditioning increased starch gelatinization from 6.97 to 13.11%. Lund & Lorenz (1984) reported that water-to-starch ratio influences gelatinization temperature. In their study, as water/starch ratio increased from 0.8 to 3.0, the onset and peak temperature for rice starch gelatinization decreased from 74.3 and 81.5 °C to 71.3 and 77.2 °C, respectively. Coral *et al.* (2009) and Fouhse (2011) reported that starch gelatinization may be influenced by grain size because it affects heat and moisture conduction across the particle. Also, the level of fat added to the diet may influence starch gelatinization rate (Moritz *el al.*, 2001).

The amylose leached from the granules act as an adhesive element between the feed particles and these binding properties of gelatinized starch have a positive effect on pellet quality (Svihus *et al.*, 2008). Moreover, the gelatinization of cereal starch may improve animal performance because the enzymatic access to glucosidic bonds may be enhanced by the disruption of the crystalline structure of starch, resulting in better digestibility (Holm *et al.*, 1988; Moritz *et al.*, 2005; Tran *et al.*, 2007). Holm *et al.* (1988) reported a 0.96 correlation between starch gelatinization and digestibility for pure starch.

Some feed-processing strategies enhance starch gelatinization and consequently, pellet quality. It is important to evaluate the effectiveness of these alternatives for changing starch structure in animal diets. The productivity and profitability of the broiler industry may improve by the correct application of these strategies. The objective of the present study was to evaluate the impact of particle size, added moisture during conditioning, dietary fat level, and the use of two different thermal processing methods on the amount of gelatinized starch (% of total starch).

MATERIAL AND METHODS

Feed processing

The experimental diets were manufactured in a broiler company feed mill located in Rio Grande do Sul State, Brazil. The feed was a broiler grower diet containing corn, soybean meal, and animal by-products with different levels of fat inclusion (15, 25, 35 and 45 g/kg of feed) (Table 1).

The ingredients were first directed to a pre-grinding sieve with a 5.0 mm diameter hole size, and all the coarse ingredients that did not pass through this screen were ground in a hammer mill (Vertical Hammer Mill with 16 hammers - 10 ton/hour output/unit x 6 units) with 5.0 mm diameter hole sieves to achieve the medium and coarse particle sizes of the finished diet. The different diet particle sizes were obtained by changing the hammer tip speed, as the hammer mill was equipped with a variable hammer rotation control (3600 rotations per minute for medium size grinding and 1800 rotations per minute for coarse size grinding). All diet components were blended in a paddle type mixer (6000-kg capacity). Mixing time was divided in three phases: dry mixing (45 seconds), liquid addition (60-90 seconds), and wet mixing (25 seconds). The different fat inclusion levels in the diet were achieved by spraying fat on the mash feed during the liquid addition phase of the mixing time.

For the conditioned-expanded-pelleted feed, diets were steam-conditioned for 15 seconds at 80-82°C under a steam pressure of 1.5-2.0 bar. The mash was then transported to an expander, with average mash retention time of 5 seconds and average temperature of 110°C (annular gap energy consumption of 11-13 k/ton/hour, 20000 mm length and 400 mm) and to the pellet press (die specifications: 660 mm diameter, 60-mm deep and 4.5-mm diameter die holes, without relief). For the simple conditioned-pelleting treatment, diets were steam-conditioned and then transported to the pellet press using the same equipment parameters that were used for the conditioned-expanded-pelleted treatment. The different levels of moisture addition in the conditioner were controlled by a water

Table 1 – Composition of the experimental diets.

Ingredients (g/kg)	15 g/kg added fat	25 g/kg added fat	35 g/kg added fat	45 g/kg added fat
Maize	672.40	662.40	652.40	642.40
Soybean meal 46% CP	235.00	235.00	235.00	235.00
Poultry by product	40.00	40.00	40.00	40.00
Fat	15.00	25.00	35.00	45.00
Feather meal	10.00	10.00	10.00	10.00
Limestone	9.50	9.50	9.50	9.50
Liquid Lysine	4.10	4.10	4.10	4.10
Salt	3.00	3.00	3.00	3.00
Liquid Methionine	2.90	2.90	2.90	2.90
Dicalcum phosphate	2.00	2.00	2.00	2.00
Sodium bicarbonate	1.50	1.50	1.50	1.50
Vitamim premix[a]	1.20	1.20	1.20	1.20
Choline chloride	0.90	0.90	0.90	0.90
Mineral premix[b]	0.85	0.85	0.85	0.85
Theonine	0.60	0.60	0.60	0.60
Phytase[c]	0.05	0.05	0.05	0.05
Total batch (kg)	1000	1000	1000	1000

[a] Supplied per kg of complete diet: 7000 IU of vitamin A. 2000 IU of vitamin D3. 25 IU of vitamin E. 2.0 mg of menadione. 4.0 mg of riboflavin. 25.0 mg of niacin. 12.0 mg of d-pantothenic acid. 4.0 mg of vitamin pyridoxin. 0.01 mg of vitamin B12. 1.0 mg of folic acid and 0.08 mg of biotin.

[b] Supplied per kg of complete diet: 10 mg of copper as copper sulfate. 1 mg of iodine as calcium iodate. 60 mg of iron as ferrous sulfate. 70 mg of manganese as manganese sulfate. 0.3 mg of selenium as sodium selenite. and 70 mg of zinc as zinc sulfate.

[c] Supplied 1000 ftu of phytase / kg of feed

proportioning system (water temperature of 60°C and water pressure of 3-6 bar). The throughput of the conditioner was used to calculate the amount of moisture added to the mash feed and moisture sensors set in the end of the conditioner were used to monitor this addition. Immediately before entering the pellet press, feeds were sampled (a pooled sample for each moisture addition level) for moisture content analysis.

Production and sampling of test feed

A total of 384 tons of feed (96 batches of 4 tons) were manufactured in this experiment. The four main factors (two particle sizes, four moisture addition levels, four fat inclusion levels, and two thermal processes) were combined in a 2 x 4 x 4 x 2 factorial arrangement in a randomized block design consisting of three production series, totaling 64 different combinations. These combinations were repeated in three production series distributed along the experimental period. The ingredients were first ground in two different particle sizes (coarse and medium) and then combined in four different fat inclusion levels in the mixer (fat added at 15 to 45 g/kg of feed - Table 1). These combinations of mash feed were transported to the conditioner, where batches were submitted to different moisture addition levels (0, 7, 14, and 21g/kg of feed) and thermal processing methods (conditioning-pelleting and conditioning-expansion-pelleting).

The sampling points were: 1) at mixer discharge: a pooled sample of mash feed was collected per treatment and production series for particle size, moisture content, and chemical composition analyses, 2) prior to the pellet press: a pooled sample of conditioned and conditioned-expanded feed was collected per treatment and production series for moisture content monitoring, and 3) between the pellet press and the cooler: three samples of pelleted feed (one sample per production series), corresponding to three replicates, were collected for starch and gelatinized starch contents determination. These pelleted feed samples were cooled under environmental conditions for 24 hours before the chemical analysis.

Average environmental temperature and relative humidity recorded during the experimental period were 24°C and 62%, respectively.

Feed analyses

Feeds from the different treatments were analyzed for the following chemical parameters: a) amount of gelatinized starch (% of total starch): determined according to Method 27 of Compêndio Brasileiro de Nutrição Animal (2009), b) moisture content: determined according to Method 930.15 of the Association of Official Analytical Chemists International (AOAC, 1998), and c) particle size: determined according to the method of the American Society of Agricultural and Biological Engineers (ASABE, 2006).

Statistical analysis

The statistical model included particle size, type of thermal processing, levels of fat and moisture addition, and interactions between factors: Yijklm = μ PRh+ PSi + TPj + FAk + MAl + (PS x TP)ij + (PS x FA)ik + (PS x MA) il +(TP x FA)jk + (TP x MA)jl + (FA X MA) kl + (PS x TPx FA)ijk + (PS x TP x MA)ijl + (PS x FA x MA) ikl + (TP x FA x MA)jkl + (PS x TP x FA x MA)ijkl + εhijklm.

Where: Yhijklm = amount of gelatinized starch (relative to the total starch content of the feed), μ = the population mean, PRh = the production series effect, PSi = effect of particle size (i = medium or coarse particle size), TPj = effect of thermal processing (j = conditioned-pelleted or conditioned-expanded-pelleted), FAk = effect of fat inclusion (k = 15 to 45 g/kg of feed), MAl = effect of moisture addition (l = 0 to 21 g/kg of feed), and εijklm = residual error.

A general linear model was employed to analyze the effects of categorical and quantitative factors present in the statistical model. Analysis of variance and multiple regression tools of Statgraphics Centurion XVI (Stat Point Technologies, Inc.) and Statistica 8.0 version (StatSoft, Inc.) were used to perform the analyses of the collected data. Mean values of the collected data are presented as least square means. The Anderson-Darling test from Minitab version 16 was used to check the normality of the residuals of the estimated model for the starch gelatinization. According to Razali & Wah (2011), the Shapiro-Wilk test and Anderson-Darling test are the most powerful normality test, followed by Lilliefors test and Kolgomorov-Smirnov test. The Hartley F-Max test was used to check the homoscedasticity of the categorical factors data (thermal processing and particle size). Linear and quadratic effects of moisture and fat addition to the feed were determined by multiple regression associated with backward elimination method. The factors and their interactions (PS x TP, PS x FA, PS x MA, TP x FA, TP x MA, FA x MA, PS x TP x FA, PS x TP x MA, PS x FA x MA, TP x FA x MA, PS x TP x FA x MA) were excluded from the final regression equation when $p>0.05$. The factors particle size and type of thermal processing were included in the regression as dummy variables (PS = 1 when medium, 0 when coarse; and TP = 0 when conditioned-pelleted, 1 when conditioned-expanded-pelleted). The significance of the performed tests were accepted when $p \leq 0.05$.

RESULTS

The residuals of the amount of gelatinized starch (% of total) did not have normal distribution (Anderson-Darling test, $p<0.05$). Yeo & Johnson (2000) reported a type of Box-Cox transformation that was capable of reducing skewness and to approximate data to normality. In the present study, this procedure, known as Johnson transformation, was used to fit the amount of gelatinized starch to Gaussian distribution: amount of gelatinized starch (% of total) = –1.10121 + 1.02935*Ln[(amount of gelatinized starch % of total - 16.2726)/(71.1461 - amount of gelatinized starch as a % of total starch)]. The transformed data were then analyzed by the Anderson-Darling test to verify the normal distribution of the data ($p>0.05$). All statistical analyses were performed using the transformed data. The Hartley's test confirmed that the variances of the amount of gelatinized starch (%) were similar among treatments (F-max was smaller than F-critical at a 0.05 significance level). In the present study, after applying the backward elimination method to the entire model, the only significant interaction found among factors was related to particle size and thermal processing. The resultant models presented R^2 values close to that of the entire model for the amount of gelatinized starch (0.725 and 0.718, respectively, for the entire and backward models).

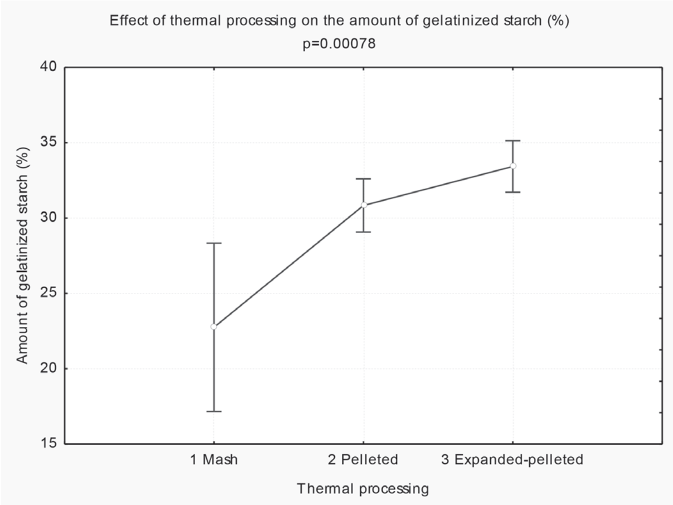
Particle size of the test diets were 1041 microns for coarse grinding and 743 microns for medium grinding, respectively. Average moisture content of the mash feed samples prior to conditioning was 102 g/kg of feed. The feed samples collected before the pellet press confirmed that moisture addition at the conditioner (0, 7, 14, and 21 g/kg of feed) increased feed moisture content prior to pelleting to 130, 138, 145 and 151 g/kg of feed, on average. The moisture content of the samples collected at the discharge of the pellet press was 7 g/kg higher for the conditioned-expanded feed than for the conditioned feed (146 and 139 g of moisture/kg of feed respectively). Data were initially analyzed according to the original statistical model. Therefore, the factor's coefficients, p-value of the model (p<0.01), and the R^2 (0.728) values of the entire model were defined in a full factorial design: 2 particle sizes x 2 thermal processes x 4 moisture addition levels x 4 fat inclusion levels (Table 2). The backward elimination method was employed to remove non-significant factors from the prediction equations (Table 3). The resulting model presented R^2 value of 0.715 and p-value <0.01.

Table 2 – Effect of thermal processing (TP), particle size (PS), moisture addition (MA), and fat inclusion (FI) on the amount of gelatinized starch

Factors	Amount of gelatinized stach (%)	P value/Effect
Thermal procesing		
Pelleted	31.98	<0.001
Expanded-Pelleted	35.33	
Particle size		
Medium	30.15	<0.001
Coarse	37.15	
Moisture addition (g/kg)		
0	31.60	<0.001 Linear effect
7	32.38	
14	35.20	
21	35.43	
Fat inclusion (g/kg)		
15	26.10	<0.001 Quadratic effect
25	37.05	
35	44.86	
45	26.60	
Interactions Probabilities		
PS x TP		0.067
PS x MA		0.847
PS x FI		0.081
TP x MA		0.152
TP x FI		0.225
MA x FI		0.567
PS s TP x FI		0.400
PS x MA x FI		0.130
TP x FI x MA		0.203
PS x TP x FI x MA		0.072
R^2		0.733
p		<0.001
SEM		4.723

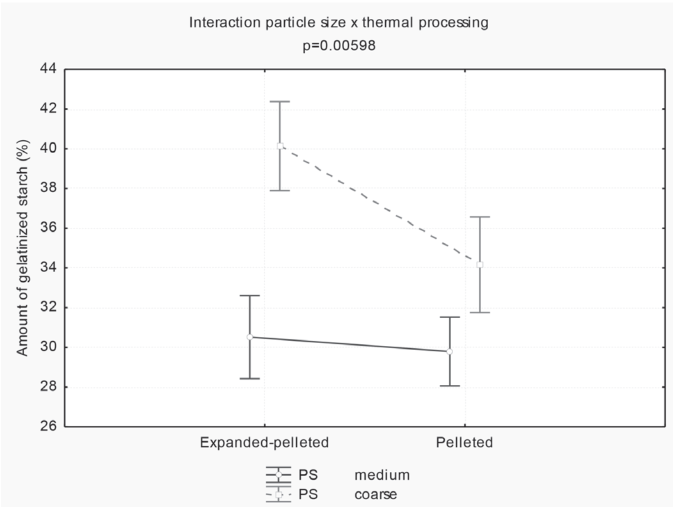
Average starch content of the samples was 502.0 ± 24.4 g/kg of feed. The samples of mash feed collected at the mixer discharge presented 22.7% gelatinized starch, on average, which was further increased (p<0.01) by the thermal processing (figure 1). Differences in starch gelatinization rate were detected between the two thermal processes evaluated in the present study: conditioning-expanding-pelleting the feed resulted in higher (p<0.01) amount of gelatinized starch (35.3%) compared with the conditioned-pelleted feed (32.0%). Also, thermal processing interacted with particle size relative to the amount of gelatinized starch. In the conditioned-expanded-pelleted feed, increasing particle size from 743 to 1041 microns increased the amount of gelatinized starch from 30.5 to 40.1%. In the conditioned-expanded feed, the same increase in particle size resulted in a smaller change of starch gelatinization degree (from 29.8 to 34.2%).

Figure 1 – Effect of thermal processing on the amount of gelatinized starch (%).



The amount of gelatinized starch in the feeds linearly increased (p<0.01) with increasing moisture addition in the conditioner (Table 3). The data obtained in the present study show a change of 3.8% of starch gelatinization (31.6 to 35.4 % of gelatinized starch) as the water addition in the conditioner increased from 0 to 21 g/kg of feed.

Figure 2 – Interaction of thermal processing and particle size on the amount of gelatinized starch (%).



Fat inclusion had a quadratic effect on the amount gelatinized starch. The inclusion of 15, 25, 35, or 45 g of fat/kg of feed resulted in 26.1, 37.1, 44.9 and 26.6% starch gelatinization, respectively (Table 2).

Table 3 – Regression equation for the amount of gelatinized starch (%) submitted to Box-Cox transformation and adjusted by the backward elimination method.

Amout of gelatinized starch (Box Cox transformtion)	Y = -4.6130019 - 0.31689749*PS + 0.577617009*TP + 0.339560758*Fl(g/kg) + 0.017268101*MA(g/kg) - 0.00558161*Fl(g/kg)^2 - 0.54385216*PS*TP	
R^2	0.715	
p	<0.001	
SEM	0.631	
Coeficients	probability	standard error
PS	<0.01	0.042
TP	<0.01	0.047
Fl	<0.01 (linear effect)	0.047
Fl^2	<0.01 (quadratic effect)	0.001
MA	<0.01 (linear effect)	0.006
PS*TP	<0.01	0.047

PS = 1 when medium, 0 when coarse
TP = 0 when conditioned-pelleted, 1 when conditioned-expanded-pelleted

DISCUSSION

Based on the enzymatic assay employed in this study, it was observed that 22.7% of the total starch feed content (502.0 +/- 24.4 g starch/kg of feed) was already gelatinized in the mash feed before thermal processing. Probably some of the ingredients used in the experimental diet had previously experienced some heating (e.g., hot air drying, grinding and solvent extraction) that caused starch gelatinization. It is currently not unusual to find industrial dryers that dry corn kernels with air heated above 120°C (Malumba *et al.*, 2009). Malumba *et al.* (2010) and Yang *et al.* (2011) reported that heat treatment using hot air above 80°C lead to starch granules swelling and weaken Maltese crosses, suggesting the occurrence of starch pre-gelatinization.

The diets submitted to conditioning-expanding-pelleting presented higher starch gelatinization values (32.0 *vs*. 35.3%) compared conditioning-pelleting. Similar results were obtained by Goelema *et al.* (1998), who submitted a mixture of broken peas, lupins, and faba beans to different thermal processes and verified that conditioning-pelleting and conditioning-expansion-pelleting resulted in 19 and 22% starch gelatinization, respectively. Svihus & Zimonja (2011) and Prestlokken & Orutvikling (2012) reported that expander treatment usually gelatinizes starch to a high extent, but the small amount of water present in the feed may limit starch gelatinization.

Moisture addition had a linear and positive effect on the amount of gelatinized starch. Moritz *et al.* (2001) also reported that increasing moisture content of the mash feed from 6.97 to 14.47 % increased starch gelatinization from 6.97 to 13.11%. This positive effect of moisture content on starch gelatinization can be partially explained by the fact that a water:starch ratio of about 1.5:1 is required for complete gelatinization (Lund & Lorenz, 1984). The linear increase in starch gelatinization rate with different moisture addition levels was probably the cause of the linear enhancement of pellet quality reported by Muramatsu *et al.* (2013). The authors showed an increase of 101 g of pellets/kg of feed, or 14% more pellets as water addition in conditioner increased from 0 g/kg to 21 g/kg of feed. Water addition to the diet, although it is not enough to achieve the ideal ratio for complete starch gelatinization, results in a more favorable water:starch ratio for starch granule hydration, swelling, and rupture.

Lund & Lorenz (1984) reported that finer particle sizes enhance starch gelatinization. Coral *et al.* (2009) and Fouhse (2011) also mentioned that smaller grain sizes may optimize the gelatinization process. Smaller particle size increases heat and water diffusion from the outside to the inside of the particle, thereby enhancing starch gelatinization. However, an opposite behavior was observed in the present study, where the degree of starch gelatinization increased as particle size increased. A possible hypothesis is that the coarser particles lead to stronger heat and frictional forces inside the pellet die, which in turn boosted starch gelatinization.

There was a positive effect of increasing fat inclusion levels on starch gelatinization. It was expected that fat addition would impair starch gelatinization as fat acts as a lubriant between the feed and the pellet die, reducing frictional heat and increasing the flow rate through the pellet press (Moritz *el al.*, 2001). Moritz *et al.* (2002) reported that the negative effect of fatty acids on starch gelatinization could be a consequence of fatty acids complexing with amylose, consequently repressing its swelling and solubilization. In the present study, the negative effect of fat on the degree of starch gelatinization was only observed when fat inclusion was increased from 35 to 45 g/kg.

This study suggests that conditioning-expanding-pelleting of diets increases (p<0.05) starch gelatinization rate compared with conditioning-pelleting. In addition, increasing moisture addition to the diet from 0 to 21 g/kg and coarse grinding had a positive effect on the amount of gelatinized starch. On the other hand, fat inclusion levels higher 35g/kg significantly decreased the content of gelatinized starch.

ACKNOWLEDGMENTS

The authors thank Lanes Tramontina, Valter Vivan, Valter Sbaraini, and João Carlos Barato for their contribution and input to this study. This research was made possible by the support of Fabrício da Silva Delgado and Luiz Stabile Benício.

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