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Thermal and Pasting Properties of Citric Acid Supplemented Poultry Diets

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Abstract: The effect of Citric Acid (CA) addition on certain thermal and pasting properties of poultry ration was investigated using modulated DSC and Rapid Visco Analyzer (RVA) techniques. The control diet was prepared based on NRC recommendations while the others were supplemented with CA at levels of 12.5, 25 and 50 g kg^{-1}. The modulated DCS results showed that acidity level affected the energy requirements for swelling and gelatinization processes. Enthalpy (ΔH), heat capacity (Cp), initial (To) and maximal (Tp) gelatinization temperature decrease as CA concentration increases in the ration. Moreover, the degree and rate of conversion for starch gelatinization was faster as the CA concentration increased. Viscoamylographic analysis also confirms that the addition of CA significantly affected the temperature at initial viscosity increase (Ti), the Peak of Viscosity (PV) and setback of the suspensions. From these results, it was concluded that poultry diets are better used when CA is added, since energy requirements decreased as CA concentration increased. Consequently, CA can be used as an additive to improve feed efficiency, animal health as well as to promote growth performance in young broiler chickens.

Key words: Poultry ration, citric acid, modulated DSC, rapid visco analyzer, broiler, health

INTRODUCTION

Sorghum is considered the fifth most important crop in the world after wheat, rice, maize and barley due to its resistance to drought and high temperatures. Its nutritional value is similar to maize and it has been used as an ingredient for inclusion in foods and feeds. Since feed represents >70% of the operating costs of intensive poultry production (Etches, 1998), special attention has been given to nutrition. For this reason, the poultry industry is continuously searching for additives to improve feed efficiency and animal health among these compounds, organic acids are promising alternatives. Dietary organic acids and their salts inhibit microorganism growth in feed and maintain the microbial balance in the Gastrointestinal Tract (GIT). Several researchers have reported positive effects of certain organic acids in poultry diets, since acidifiers might improve poultry performance by reducing colonization of pathogenic microorganisms and toxic bacterial metabolites such as ammonia and amines (Chaveerach *et al*., 2004).

Citric Acid (CA) is one of the most widely-used food additives which is commonly used as a preservative, acidifier, pH control agent, flavor enhancer and antioxidant. Global production (mainly through microbiological fermentation) is estimated to be approaching 4×10^5 tons $year^{-1}$ (Kristiansen *et al*., 1999).

With the introduction of sensitive instrumentation in the last decade, thermal analysis techniques have rapidly gained prominence in food/feed research. Recent investigations have highlighted the applications of thermal analysis in studying a broad variety of food systems. Not only the fundamental structure-property relationships of the separate ingredients are studied but also the complex behavior of complete food products.

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One of the most popular thermal analysis techniques is Differential Scanning Calorimetry (DSC). Interpretation of heat flow curves of DSC thermograms however is often difficult due to the complex nature of real food/feed products. With modulated DSC, a recent extension of conventional DSC using a modulated temperature input signal, information on the amplitude of the complex heat capacity is obtained both in quasi-isothermal and non-isothermal conditions. This complementary information giving rise to a deconvolution of the (total) heat flow signal into reversing and non-reversing contributions enables a more detailed study of complicated material systems. While modulated DSC has proven to be beneficial for the thermal characterization of synthetic polymers in general this relatively new technique has not yet been extensively applied to food systems.

The recent studies indicated that aqueous CA degrades aflatoxins (a group of acutely toxic metabolites produced by toxigenic strains of *Aspergillus flavus* Link, *A. parasiticus* Speare and A nomius Kurtzman in the ration (Mendez-Albores *et al*., 2009). Considering that the acidic treatment protects ducklings from chronic aflatoxin toxicity (Mendez-Albores *et al*., 2007) and improves growth performance in young broiler chickens (Salgado-Transito *et al*., 2011), the purpose of the present study was to determine the effect of the CA addition on certain thermal (gelatinization enthalpy, heat capacity, rate and degree of conversion and transition temperatures) and pasting (onset temperature, viscosity peak and setback) properties of acidified poultry rations.

MATERIALS AND METHODS

Chemical: Anhydrous citric acid (99.9% purity) was obtained from Mallinckrodt Baker (JT Baker, Xalostoc, Mexico). The chemical properties of citric acid are as follows: chemical formula $C_6H_8O_7$ and molecule weight 192.13 g mol^{-1}.

Grain sorghum: Grain sorghum (*Sorghum bicolor* L. Moench) of the commercial variety RB-3030 with initial Moisture Content (M.C.) of 9.7% was utilized. M.C was determined by drying replicate portions of 5-10 g each of whole grain at 103°C for 72 h with concentrations calculated on a wet-weight basis.

Experimental diet: A control sorghum-soybean based diet was prepared on NRC (1994) recommendations. For preparing other treatments, the control diet was supplemented with CA at levels of 6.25, 12.5, 25 and 50 g kg^{-1} of diet, respectively. The composition and chemical analysis of the diet are shown in Table 1. No antibiotic or anticoccidial drug was used in any diet and rations were prepared so as to be isocaloric and isonitrogenous. The average Moisture Content (M.C.) of the rations was 12%. The pH of the rations was determined according to the 02-52 AACC method (AACC, 2000).

Table 1: Ingredients and nutrient composition of the control experimental diet

Ingredients	Percentage	Calculated analysis[4]	Percentage
Sorghum	50.33	ME (kcal kg^{-1})[5]	3010.00
Soybean meal (48)	41.52	Crude protein	24.00
Sunflower oil	3.46	Calcium	1.00
Orthofosfate	1.86	Phosphorus, total	0.50
Calcium carbonate	1.56	Methionine+Cystine	1.60
Salt (NaCl)	0.41	Lysine	1.50
Alimet 88[1]	0.35	Threonine	0.97
L-Lisine HCl	0.19	Zinc (mg kg^{-1})	45.00
Choline chloride	0.10	-	-
Vitamine premix[2]	0.10	Analyzed	-
Mineral premix[3]	0.05	Crude protein	23.75
Sugar+zinc	0.05	Calcium	1.10
L-Threonine	0.03	Phosphorus, total	0.57

[1] Aqueous solution of 2-Hydroxy-4-(Methylthio) Butanoic Acid (HMTBA). [2] Vitamine premix supplied kg^{-1} diet: Vitamin A, 12 000 IU; Vitamin D_3, 2,500 IU; Vitamin E, 15 mg; Vitamin K_3, 2 mg; Vitamin B1, 2.25 mg; Vitamin B2, 7.5 mg; Vitamin B6, 3.5 mg; Vitamin B12, 0.020 mg; folic acid, 1.5 mg; pantothenic acid, 12.5 mg. [3] Mineral premix supplied/kg diet: Cu, 8 mg as $CuSO_4 5H_2O$; Mn, 100 mg as MnO; Fe, 80 mg as $FeSO_4 H_2O$; I, 1 mg as Ethylenediamine Dihydroiodide (EDDI); Se, 0.15 mg as Na_2SeO_3. [4] Data on dry matter; [5] ME: Metabolizable Energy

Modulated DSC measurements: Samples were analyzed using a differential scanning calorimeter equipped with a modulation extension apparatus (DSC 2920, TA Instruments, New Castle, USA). Cooling was carried out with a refrigerated cooling system (TA Instruments, New Castle, USA). The temperature calibration was performed using TA instruments software with indium (melting point value of 156.6°C, standard TA instruments, New Castle, USA). The heat capacity was calibrated with sapphire (aluminum oxide). The TA instruments universal analysis software was used to record and analyze the thermograms. Samples and water at a ratio of 1:1 (w/w) were packed down in hermetic aluminum DSC pans (TA Instruments, New Castle, USA); pans were allowed to equilibrate at room temperature (22±2°C) for at least 1 h before testing. The DSC was operated in modulation mode. Samples were analyzed in triplicate by heating in the modulated DSC furnace in atmosphere of nitrogen at a rate of 5°C min^{-1} with temperature modulation of 0.8°C/60 sec. Thermal decomposition data for the samples were collected over the temperature range 20-150°C.

Additionally, the degree (α) and the conversion rate ($d\alpha/dt$) for starch gelatinization were computed as follows:

$$\alpha = \frac{\Delta H_P}{\Delta H_T}$$

$$\frac{d\alpha}{dt} = \frac{\left(\frac{\Delta H_P}{dt}\right)}{\Delta H_T}$$

where, ΔH_p, ΔH_T = Partial and total enthalpy (J/g), respectively.

Pasting properties: Relative viscosity of water suspensions of the diets was determined in a Rapid Visco Analyser RVA-4 (Newport Scientific, Sydney Australia). A sample 3.5 g (60 mesh) at 14% M.C was suspended in 25.5 g of distilled water. A plastic stirring paddle was placed in the sample recipe which was then fixed into the RVA and the heating cycle was activated through a split copper block. The analyzer used a time-temperature program as follows: initiating at 50°C, increasing the temperature until 90°C at a speed of 8.0°C min^{-1} (5 min), remaining 5 min to that temperature and later diminishing the temperature to 50°C at the same speed used during the heating and remaining at that temperature during 1 min with a total test time of 16 min. The rotating speed of the paddle was 860 rpm for the first 10 sec then 160 rpm for the remainder of analysis. From the pasting curves, the temperature at initial viscosity increase (T_i), temperature at peak viscosity (T_p), Peak Viscosity (PV) and setback (difference between the viscosity at the end and the beginning of the cooling period) were registered. Three replicates per sample were analyzed.

Experimental design and statistical analysis: Data were assessed by Analysis of Variance (ANOVA) and means were separated by the Dunnet procedure using the Statistical Analysis System (SAS, 1998). A significance value of (α = 0.05) was used to distinguish significant differences between treatments.

RESULTS AND DISCUSSION

Experimental diet acidification: Table 1 shows the ingredients and nutrient composition of the control experimental poultry diet. The pH of the diet declined according to the amount of CA added. Ration with no acid (control) presented an average pH value of 6.2 while rations added with CA at concentrations of 12.5, 25 and 50 g kg^{-1}, presented pH values of 5.1, 4.5 and 3.9, respectively. The reasons of the use of CA in poultry rations are that organic acids are extremely efficient against bacteria in poultry because CA possesses inhibiting, bacteriostatic and neutralizing effects against ammonifying bacteria, *E. coli* and Salmonellae (Ivanov, 2001); CA also have a positive effect on growth performance; since dietary acidification increases gastric proteolysis and protein/amino acid digestibility by enhancing digestive enzyme activities (Langhout, 2000). Moreover, CA removes calcium from plant based phytate complexes thereby allowing the phytate to solubilize making the phosphorus contained in the phytate complexes bioavailable. In addition, CA also increases utilization of the phosphorus from inorganic sources in the diet thus further reducing the need for supplemental inorganic phosphorus among others.

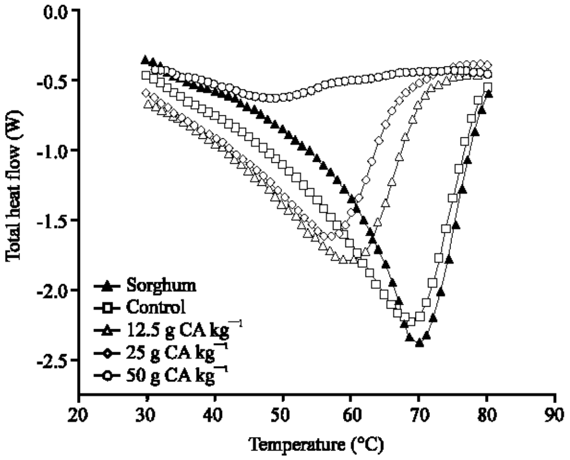

Fig. 1: Typical modulated DSC thermograms of sorghum and acidified ration

Table 2: Gelatinization parameters for sorghum and acidified poultry ration

	Gelatinization endotherm			
g CA kg^{-1}	To (°C)	Tp (°C)	Tc (°C)	ΔH (J g^{-1})
Sorghum	56.07^a	69.88^a	83.53^a	414.18^a
0 (control)	44.39^b	67.58^a	82.62^a	360.93^b
12.5	39.43^c	63.53^b	82.08^a	321.60^c
25	35.39^d	56.89^c	77.84^b	215.80^d
50	35.11^d	48.17^d	69.35^c	39.49^e

Mean of three replicates±standard error; mean values with same letter in the same column are not significantly different (Dunnet>0.05); To, Tp, Tc: Onset, peak and conclusion temperatures; ΔH: Enthalpy of gelatinization

Modulated DSC (total heat flow): The typical modulated DSC thermograms of the CA added rations, the control diet as well as raw sorghum are shown in Fig. 1. The gelatinization transition temperature [To (onset), Tp (peak) and Tc (conclusion)] and the enthalpy of gelatinization (ΔH) are shown in Table 2. In this research, all samples presented similar modulated DSC curves however, in the CA added samples a displacement in the maximum gelatinization temperature was observed as compared to those of control diet and raw sorghum. The shapes of the sorghum endotherms corresponded closely with those reported previously (Sang *et al*., 2008) with raw sorghum having an endotherm almost identical with that of the control diet. On the other hand, the raw sorghum and the control diet presented average To values of 56.07 and 44.39°C, respectively. In the case of acidified diets as the CA concentration increase, lower To values were registered. The same phenomenon was observed in the case of Tp; sorghum samples presented a Tp value of 69.88°C while in acidified diet with 50 g CA kg^{-1} the Tp was 48.17°C. Sorghum starch gelatinization temperature ranges of 67-73°C have been reported for sorghum grown in South Africa and 71-81°C for sorghum grown in India (Taylor *et al*., 2006). Starch gelatinization temperature is influenced by many factors; in particular the lengths of the various chains in the amylopectin molecule with gelatinization temperature increasing with longer chain length. It is well known that each type of starch gelatinized at a different temperature range and that sorghum starch gelatinizes between 66 and 77°C (Rooney and Serna-Saldivar, 1987). Consequently, the differences in Tp could be explained largely by variations in genetic and environmental factors. In this research, CA addition significantly affected the gelatinization temperature; probably, acidification caused a decrease in the Tp (about 19°C) due to modifications on the starch granules (Table 2). Regarding the Tc values; no statistical differences were observed between sorghum, control diet and the CA added diet (12.5 g CA kg^{-1}); those samples presented an average value of 83.53, 82.62 and 82.08°C, respectively. However, in the case of samples added with 25 and 50 g CA kg^{-1}, lower Tc values were registered. Considerable differences were also observed for the enthalpy of gelatinization (ΔH) for sorghum, control and acidified samples. As the CA concentration increase, lower ΔH values were registered. Samples with no acid (control) presented an average value of 360.93 J g^{-1}, different to those found for sorghum (414.18 J g^{-1}) while the lowest ΔH value (39.49 J g^{-1}) was observed in samples prepared with 50 g CA kg^{-1} (Table 2). Moorthy (2002) reported that gelatinization enthalpy depends on number of factors such as crystallinity intermolecular bonding and it also depends on genetic and environmental factors.

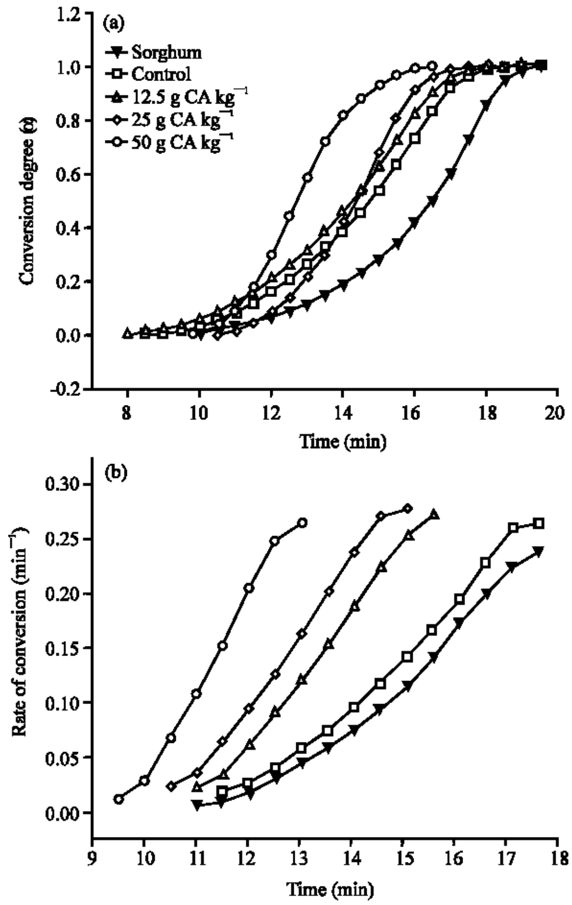

Fig. 2: Plot of the conversion degree (a) and rate of conversion (b) for sorghum and acidified ration

Degree and rate of conversion: The degree and rate of conversion was used to observe the combined effect of CA concentration, temperature and time over the gelatinization; considering that the gelatinization shows a first order irreversible reaction (Turhan and Gunasekaran, 2002). Therefore, the kinetic parameters were derived of non-isothermal experiments according to recommendations of Spigno and Faveri (2004). Considering that the temperature in the maximum deflection is the temperature of the maximum reaction rate. Figure 2 shows the results for the conversion degree and the conversion rate for the starch gelatinization in acidified ration. It is observed that when used a heating rate of 5°C min^{-1}, the conversion degree (Fig. 2a) is an important function of the acidity level thus the complete gelatinization conversion is faster in samples added with 50 g CA kg^{-1} than the control therefore, reducing the reaction time in 2.2 min, approximately. Figure 2b also confirms this behavior; samples added with 50 g CA kg^{-1} finished the gelatinization process at 13 min with a conversion rate of 0.27 min^{-1} on the contrary, samples with no acid (control) finished gelatinization at 16 min with a conversion rate of 0.195 min^{-1}. These results showed that the inclusion of CA in the ration, promotes an earlier solubilization of the sorghum starch.

Heat capacity: Another important factor to visualize structural changes in materials is heat Capacity (Cp). Figure 3 shows the behavior of the Cp; continuous

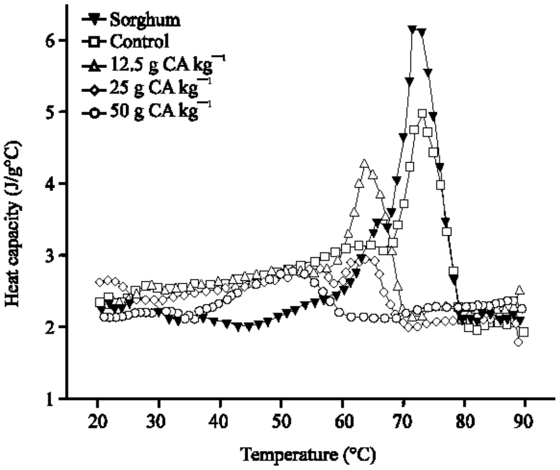

Fig. 3: Heat capacities of sorghum and acidified ration

changes were observed in the range of 30-80°C. As the CA concentration increase, lower values in the Cp were observed. Sorghum presented the highest Cp value (6.15 J/g°C) at 72°C. The result is consistent with the value reported by Gennadios *et al.* (1994) for sorghum grain with moisture content between 8.6-16.3% (wet basis). Samples with no acid (control) presented a maximum Cp value of 4.95 J/g°C at 73°C. However, samples added with 12.5, 25 and 50 g CA kg^{-1} presented average Cp values of 4.25, 2.97 and 2.83 J/g°C, respectively. Those values were observed in the temperature range of 51-64°C. In general, heat capacity values were always lower in acidified samples. Several studies have used DSC to measure heat capacity of starch during heating. Liu and Lelievre (1991) investigated changes in heat capacity of starch suspensions in association with melting transitions. Hwang *et al.* (1999) reported changes in the heat capacity of corn starch due to gelatinization. However, none of the reported studies have investigated the influence of acidifiers in feed. In this research, researchers hypothesized that the decrease in the Cp was associated with changes in molecular structure due to the CA addition.

Viscoamylographic properties: When starch granules are heated in water excess, granules irreversibly swell and lose their native birefringence and crystalline order and form a paste. In contrast, pasting is the phenomenon following gelatinization, involving granular swelling, exudation of molecular components from the granule and eventually, total disruption of the granule (Atwell *et al.*, 1988). The viscoamylographic profiles are shown in Fig. 4 shows the greater swelling power of sorghum (A) compared to ration (B). In general, CA addition significantly reduced the peak viscosity in the acidified diet however, all samples showed the same viscoamylographic profile. Viscoamylographic properties are shown in Table 3. There were statistical differences in the temperature at initial viscosity increase (Ti) between sorghum and the ration. The Ti gelatinization value for sorghum was 42.27°C however, an increment of about 17°C was observed in the case of the rations. Subramanian *et al.* (1994) reported higher Ti values (69-77°C) for seven sorghum starches. The differences in swelling temperatures possibly resulted from genetic variation in kernel structure of the varieties, considering that most Zimbabwean sorghums having an intermediate to floury endosperm.

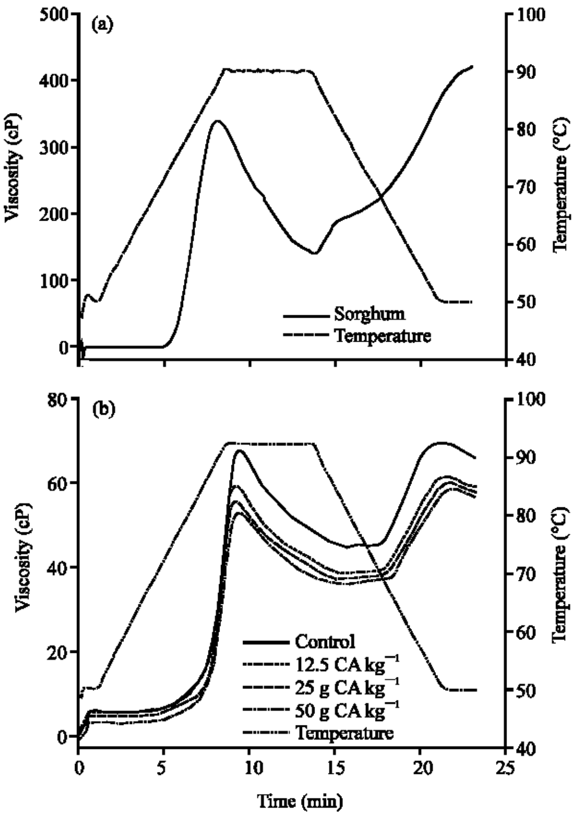

Fig. 4: Viscoamylographic curves of sorghum (a) and acidified ration (b) measured by Rapid Visco Analizer

Table 3: Viscoamylographic characteristics of sorghum and acidified diets

	Viscoamylographic parameters			
g CA kg^{-1}	Ti (°C)	Tp (°C)	PV (cP)	Setback (cP)
Sorghum	42.27^{a}	81.36^{a}	339.00^{a}	233.83^{a}
0 (control)	58.36^{b}	90.90^{b}	67.44^{b}	20.96^{b}
12.5	59.35^{b}	84.28^{a}	58.65^{c}	19.64^{b}
25	59.70^{b}	82.27^{a}	55.32^{cd}	19.31^{b}
50	59.65^{b}	80.13^{a}	52.71^{d}	19.14^{b}

Mean of three replicates±standard error; mean values with same letter in the same column are not significantly different (Dunnet>0.05); Ti, Tp: Temperature at initial and peak viscosity PV: Peak Viscosity

Regarding the temperature at peak viscosity (Tp); sorghum presented an average Tp value of 81.36°C. On the contrary in the control and acidified samples, the Tp value was in the range of 90.90-80.13°C. Consistently as the CA concentration increases lower Tp values were found (Table 3). On the other hand, the mean Peak Viscosity (PV) of sorghum (339.0 cP) was markedly higher than that of control and acidified diet. Thus sorghum had a greater water-binding capacity than the ration. The results are in close agreement with those of Boudries *et al*. (2009) who reported PV values of 341.08 and 394.25 cP for white and red sorghum, respectively. In this research, statistical differences in the PV were observed due to the addition of CA in the ration as the CA concentration increased, lower viscosity values were registered. Samples with no acid (control), presented an average PV value of 67.44 cP. The lowest PV value (52.71 cP) was observed in samples prepared with the addition of 50 g CA kg^{-1} (Table 3). It is most likely that extreme pH values are a contributing factor to starch degradation, resulting in lower viscosity as previously reported in maize starch acidified with lactic acid (Haros *et al*., 2004).

When cooled, pastes will form a viscoelastic gel; the molecular reassociation that occurs during the cooling and storage of gelatinized starch molecules to form an ordered structure are defined as starch retrogradation or setback. Thus setback is an important factor in some food-processing operations. Sorghum was the sample that presented the highest setback value (233.83 cP). Additionally, there were no significantly differences in the setback for the control and the acidified samples however, as in the case of PV, lower values were registered as the CA concentration increase. Samples with no acid (control) presented an average setback value of 20.96 cP and the lower setback value (19.14 cP) was observed in samples prepared with the addition of 50 g CA kg^{-1} (Table 3). Sang *et al*. (2008) reported that sorghum starches differing in amylose content at pH of 3, displayed reduced peak viscosity and increased breakdown compared with neutral pH. The same trend was observed in this research however, the results cannot be compared because different sorghum sources (nonisogenic waxy, heterowaxy and normal sorghum hybrids) and type of acidifier (sodium citrate buffer) were used. Starch setback is influenced by the fine structure of amylopectin and the amylose/amylopectin ratio (amylose setback occurring at a faster rate than that of amylopectin) also the water content in starch, storage temperature and syneresis can affect the rate and extent of starch setback (Keetels *et al*., 1996). Additionally, setback has been related to changes in the texture and digestibility of stored starch-based food/feed. Nevertheless in this study, it appears that PV and setback were more influenced by the CA addition than by other factors.

CONCLUSION

With the use of modulated DSC technique; the current investigation showed that the inclusion CA significantly reduce the enthalpy (ΔH), heat capacity (Cp) as well as the initial (To) and maximal (Tp) gelatinization temperatures in the acidified ration. Also the degree and rate of conversion for starch gelatinization was faster in samples added with more CA inclusion. RVA analysis also confirms that the addition of CA in the ration significantly affected the peak of viscosity and setback of the suspensions. However, systematic studies are needed to determine the effect of CA inclusion over other thermal, pasting, structural, functional and nutritional properties of acidified poultry diets, since these formulations promote growth performance in young broiler chickens.

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