Feed acidification and steam-conditioning temperature influence nutrient utilization in broiler chickens fed wheat-based diets

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ABSTRACT Two experiments were conducted to examine the effects of conditioning temperature (**CT**) and the interactive influence of feed acidification (**FA**) and CT on the performance, coefficient of apparent ileal digestibility (**CAID**) of nitrogen (**N**), starch, fat calcium (**Ca**) and phosphorus (**P**), and AME in broilers. In both experiments, each treatment was randomly allocated to 6 cages (8 birds per cage) and fed from 1 to 21 D post-hatch. In experiment 1, the effect of CT was evaluated using a wheat-based diet at 3 CT: unconditioned, conditioned at 60°C or 90°C. All the diets by-passed the pellet press and collected in mash form. Birds fed the diet conditioned at 90°C consumed more ($P < 0.05$) feed and tended ($P = 0.087$) to have higher feed per gain (**F:G**) than those fed the unconditioned diet but similar to those fed the diet conditioned at 60°C. A tendency was noted for CT to affect the CAID of N ($P = 0.071$) and starch ($P = 0.093$), with reduced digestibility values in the diet conditioned at 90°C. Conditioning at 90°C resulted in lower ($P < 0.05$) AME. In experiment 2, three inclusions of an acidifier (0.0, 7.0, and 10 g/kg) and 2 CT of 60°C and 90°C were evaluated in a 3 × 2 factorial arrangement of treatments using pelleted diets. Neither the main effects nor the interaction between acidifier addition and CT was significant for weight gain, feed intake, and F:G. The FA increased ($P < 0.05$) the CAID of N, fat, and P at both inclusion levels and of starch at 10 g/kg. Conditioning at 90°C reduced ($P < 0.05$) the CAID of starch, fat, and Ca, regardless of FA level. Overall, the present data showed that the application of high CT for broiler feed manufacture can impair nutrient utilization and, consequently the feed efficiency in broilers. Feed acidification imparts some benefits to nutrient digestibility in broilers fed pelleted wheat-based diets.

Key words: acidifier, broiler, conditioning temperature, nutrient digestibility

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INTRODUCTION

The last century has been marked by a rapid and extensive modernization in poultry feed processing techniques, and modern broiler chickens are fed an agglomerated high nutrient-dense feed in the form of pellets. The advantages of feeding pelleted diets to enhance feed efficiency and economics in the production of broiler chickens is unequivocal and has been the subject of numerous recent reviews (Abdollahi et al., 2013a, 2018, 2019; Goodarzi Boroojeni et al., 2016). However, while the effect of feeding pelleted diets to broilers is clearly advantageous, variability exists in performance or nutrient digestibility responses. Part of this response variation might be associated, *inter alia*, with application of high-temperature heat treatment during the pelleting process and its opposing effects on nutrient digestibility and physical pellet quality. It is well documented that the use of high conditioning temperature (**CT**) damages dietary nutrients with a consequential loss in nutrient utilization and bird performance (Kirkpinar and Basmacioglu, 2006; Abdollahi et al., 2010a,b, 2011; Loar II et al., 2014). However, the better physical pellet quality achieved at higher CT might restore the performance of broilers (Abdollahi et al., 2010a,b), depending on the magnitude of the negative effects of CT on nutrient availability.

In the poultry feed industry, high CT are employed to i) create high quality pellets and ii) eliminate feed-borne pathogens such as *Salmonella* and *Campylobacter* to meet the hygiene standards. Considering the detrimental impact of high CT on an array of major and expensive nutrients including starch, amino acids,

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vitamins, and feed additives such as exogenous enzymes and probiotics, strategies other than high CT should be practised to maintain pellet quality and to preserve the nutritional quality of the feed. Several alternative strategies such as prepelleting additions of water or pellet binder (Abdollahi et al., 2012) and longer pellet length and small diameter die hole (Abdollahi et al., 2013b) have been investigated and proven to be effective in creating high physical quality pellets at low CT. However, lowering the CT raises concern over feed sterilization and *Salmonella* elimination which is thought to require high-temperature heat treatment. While published data on the effects of temperature on feed hygiene are inconsistent (McCapes et al., 1989; Veldman et al., 1995; Jones and Richardson, 2004; van Immerseel et al., 2009; Jones, 2011), it has been suggested that pelleting process offers no protection against recontamination of feed and that the sterilizing effects of pelleting may be lost after the feed leaves the pellet press (Jones and Richardson, 2004).

The use of organic acids in poultry diets has attracted some attention over the years and their benefits on gut health, nutrient digestibility, and growth performance have been documented (García et al., 2007; Ao et al., 2009; Chowdhury et al., 2009; Adil et al., 2011; Palamidi et al., 2017). The initial motivation of using organic acids in poultry diets has been to protect the feed from microbial contamination and spoilage through acidification (van Immerseel et al., 2006), and theoretically, they should be able to reduce pH in bird's digestive tract. It has been suggested that pH reduction in the digestive tract because of organic acid inclusion may increase mineral absorption and digestibility of protein by enhancing pepsin activity (Lückstädt and Mellor, 2011). Moreover, a recent study by Jendza et al. (2018) revealed that the combination of feed acidification (**FA**) and low CT (60°C) resulted in no detectable *Salmonella*, a finding that was comparable to steam-conditioning the same diet without FA at 75°C or 90°C. This finding could indicate that the effect of FA and CT are potentially additive on feed hygiene. While conditioning at 75°C and 90°C were equally effective at increasing the hygiene of broiler feed, it offered only minimal protection against postpelleting recontamination. However, FA was effective against recontamination regardless of CT (Jendza et al., 2018).

To the authors' knowledge, no study has ever investigated the interaction between FA and CT in poultry. The present study was initiated to explore the possibility of applying lower CT in combination with FA as complementary tools for promoting feed physical quality and maximizing its nutritional value. Thus, the objectives of this study were to determine i) the effects of CT *per se* on nutrient digestibility and performance of broiler chickens using steam-conditioned mash diets and ii) whether there are interactive effects between FA and CT on the nutrient utilization and growth performance of broilers fed pelleted wheat-based diets.

MATERIALS AND METHODS

The experiments were conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing, and teaching and approved by the Massey University Animal Ethics Committee.

Diets

Feed ingredients were obtained from a commercial supplier. Whole wheat was ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a screen size of 4.0 mm to achieve medium grind. In experiment 1, a wheat–soybean meal–based diet, with no acidifier inclusion, was formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2014; Table 1). The formulated diet was mixed and then divided into 3 equal batches. One was offered as unconditioned mash diet, and the other 2 batches were passed through the conditioner chamber and steam-conditioned at either 60°C or 90°C, by-passed the pellet press, and the mash collected at the outlet of the cooling chamber.

In experiment 2, three broiler starter diets based on wheat and soybean meal, with 3 inclusions of acidifier (0.0, 7.0, and 10.0 g/kg), were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2014; Table 1) and to be equivalent in respect of energy and digestible amino acid contents. The basal diet with no acidifier was the same as that used in experiment 1. The acidifier, Amasil NA (BASF, Ludwigshafen, Germany), was in liquid form. The active ingredients of Amasil NA are formic acid (61%) and sodium formate (20.5%), with a water content of 16 to 20%, sodium content of 6 to 8% and pH (100 g/L water) of 2.6 to 3.2. The sodium content of Amasil NA was accounted for when formulating the diets. In the acidifier diets, the acidifier was added manually to the mash diet in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia). After addition, diets were allowed to mix for 7 min, followed by the addition of soybean oil and mixed for another 7 min. The diets contained 5.0 g/kg titanium dioxide (Merck KGaA, Darmstadt, Germany) as an indigestible marker for the determination of ileal nutrient digestibility. Each mixed diet was then divided into 2 equal batches, and 1 was steam-conditioned at 60°C, and the second batch was steam-conditioned at 90°C to provide a 3 × 2 factorial array of dietary treatments. All diets were pelleted using a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3.0 mm apertures and a depth of 35 mm. Pellets were of 2.0 mm length, and representative samples were collected, and pellet durability was determined.

In both experiments, steam-conditioning time of the mash was 30 s, and the CT was measured at the outlet (close to the exit point) of the conditioner before the

Table 1. Composition, calculated analysis, and analyzed values of the experimental diets (g/kg, as-fed basis).

Item	Acidifier inclusion rate (g/kg, as-fed basis)[1]		
	0.0[2]	7.0	10.0
Wheat	632.9	624.3	620.6
Soybean meal, 480 g/kg	297.7	298.9	299.5
Soybean oil	28.14	30.19	31.0
Dicalcium phosphate	9.84	9.89	9.91
Limestone	11.93	11.91	11.89
DL-Methionine	2.87	2.89	2.90
L-Lysine HCl	3.46	3.45	3.44
L-Threonine	1.13	1.13	1.14
Sodium chloride	1.42	1.43	1.43
Sodium bicarbonate	2.86	1.15	0.42
Trace mineral premix[3]	1.50	1.50	1.50
Vitamin premix[3]	0.80	0.80	0.80
Choline chloride 60%	0.25	0.26	0.27
Titanium dioxide (TiO2)[4]	5.0	5.0	5.0
Phytase[5]	0.10	0.10	0.10
Carbohydrase[6]	0.10	0.10	0.10
Acidifier	0.0	7.0	10.0
Calculated analysis			
AME (kcal/kg)	3,010	3,010	3,010
CP	219.9	219.5	219.3
Calcium	10.0	10.0	10.0
Non-phytate phosphorus	5.0	5.0	5.0
Sodium	1.8	1.8	1.8
Chloride	2.0	2.0	2.0
Potassium	8.5	8.5	8.5
Digestible lysine	12.3	12.3	12.3
Digestible methionine + cysteine	8.74	8.74	8.74
Digestible threonine	7.80	7.80	7.80
Analyzed values			
Dry matter	876	875	868
Gross energy (kcal/kg)	3,929	3,929	3,893
CP (Nitrogen × 6.25)	226.5	221.3	216.6
Starch	344	342	332
Fat	38.9	42.7	40.3
Calcium	8.4	9.3	8.7
Total phosphorus	5.4	5.6	5.6

[1]Amasil NA, BASF, Germany. Active ingredients: formic acid, 61% and sodium formate, 20.5%. Provided in liquid form with water content of 18 ± 2.0%. Sodium (Na) content of Amasil NA (67 g Na/kg) was accounted for in feed formulation.

[2]The diet with no acidifier inclusion was used in experiments 1 and 2.

[3]Supplied per kg of diet: vitamin A (trans-retinyl acetate), 12,000 IU; vitamin D3 (cholcalciferol), 4,000 IU; vitamin E (DL-α-tocopherol), 80 IU; biotin, 0.25 mg; pantothenic acid (D-Ca pantothenate), 15 mg; vitamin B12 (cyanocobalamin), 0.02 mg; folic acid, 3.0 mg; vitamin K3 (menadione nicotinamide bisulphite), 4.0 mg; niacin (nicotinic acid), 60 mg; pyridoxine (pyridoxine. HCl), 10 mg; riboflavin, 9.0 mg; thiamine (thiamine-mononitrate), 3.0 mg; antioxidant (ethoxyquin), 100 mg; choline (choline chloride 60%), 360 mg; Co (cobalt sulfate), 0.15 mg; Cu (copper sulfate), 6.0 mg; organic Cu, 3.0 mg; Fe (iron sulfate), 36 mg; I (calcium iodate), 0.93 mg; Mn (manganese oxide), 60 mg; Mo (sodium molybdate), 0.15 mg; Se (sodium selenite), 0.26 mg; organic Se (enriched yeast), 0.14 mg; Zn (zinc sulfate), 48 mg; organic Zn, 24 mg.

[4]Merck KGaA, Darmstadt, Germany.

[5]BASF Natuphos E 10,000 G (hybrid 6-phytase; 1,000 phytase units [FTU]/kg diet). One unit of phytase activity is defined as the amount of enzyme, which liberates 1.0 μmol of inorganic phosphorus per minute from a solution of 0.0051 mol/L sodium phytate at pH 5.5 and 37°C. Full nutrient matrix values for phytase were used in diet formulation.

[6]BASF Natugrain TS. One thermostable endo-xylanase unit is defined as the amount of enzyme, which liberates 5.0 μmol reducing sugars, measured as xylose equivalents per minute from a buffer solution containing 1.0 g arabinoxylan per 100 mL at pH 3.5 and 40°C. One thermostable endo-glucanase unit is defined as the amount of enzyme, which liberates 1.0 μmol reducing sugars, measured as glucose equivalents per minute from a buffer solution containing 0.714 g beta-glucan per 100 mL at pH 3.5 and 40°C.

mash feed entered the pellet die. The CT of the mash was measured continuously as a single-point measure during conditioning using a digital thermometer (Dick Smith Electronics, China). The desired CT were achieved by adjusting the steam flow rate. Natuphos E 10,000 G (hybrid 6-phytase) (BASF) at 1,000 FTU/kg feed and Natugrain TS (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) (BASF) at 100 g/tonne of feed were added across the basal diets in both experiments. Both enzymes are thermostable and were added to the diets in mixer (prepelleting enzyme addition). The activity of phytase in feed samples were measured at Vita Company Limited, Bangkok, Thailand. The phytase recovery was calculated as the percentage of measured phytase activity in the diet to the expected enzyme activity estimated from the minimum activity of phytase and the amount of phytase added to the diets. All diets were manufactured and stored for 2 wk before the start of the trial, and then, before feeding, DM content of all diets were determined to ensure that feed intake (**FI**) measurements were not biased by differences in moisture content.

Pellet Durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi et al. (2010b). Clean pellet samples (100 g; 6 replicates per diet), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 s. Fines were removed continuously through the perforations during the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The pellet durability index (**PDI**) was calculated as the percentage of the pellets not passing through the perforations at the end of the test.

Birds and Housing

In both experiments, day-old male broilers (Ross 308) were obtained from a local hatchery, individually weighed, and allocated to 18 cages in experiment 1 or 36 cages in experiment 2 in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the dietary treatments, in both experiments, was randomly assigned to 6 cages, each housing 8 birds, with total of 144 and 288 birds used in experiments 1 and 2, respectively. On day 12, birds were transferred to grower cages and continued the same diets until day 21. The space allocation per bird in brooder and grower cages was 530 and 640 cm^2, respectively. The battery brooders and grower cages, with wired floors, were housed in an environmentally controlled room with 20 h of fluorescent illumination per day. The temperature was maintained at 31°C on day 1 and decreased

by 3°C per week to a final temperature of 22°C at 21 D of age. Cages were equipped with feed troughs and nipple drinkers. Diets, in mash form in experiment 1 and pellet form in experiment 2, were offered ad libitum, and water was freely available.

Performance Data

Body weight and FI were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (**F:G**) values were corrected for the weight of any bird that died during the course of the experiment.

AME Determination

Feed intake and total excreta output for each cage were monitored from day 17 to 20 posthatch. Daily excreta collections from each cage were pooled, mixed in a blender, and subsampled. Subsamples were lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve, and stored in airtight plastic containers at 4°C pending analysis. The diets and excreta samples were analyzed for DM and gross energy (**GE**).

Determination of the Coefficient of Apparent Ileal Digestibility

On day 21, all birds per cage were euthanized by intravenous injection (1 mL per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta were collected from the lower half of the ileum, as described by Ravindran et al. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-cecal junction. The ileum was then divided into 2 halves, and the digesta was collected from the lower half toward the ileo-cecal junction. Digesta from birds within a cage were pooled, lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve, and stored at 4°C until laboratory analysis. The diets and digesta samples were analyzed for DM, titanium (**Ti**), nitrogen (**N**), starch, fat, calcium (**Ca**), and phosphorus (**P**).

Chemical Analysis

Dry matter was determined using standard procedure (Method 930.15; AOAC, 2005). Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardized with benzoic acid. Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996). Nitrogen was determined by combustion (Method 968.06; AOAC, 2005) using a carbon nanosphere-200 carbon, N, and sulfur autoanalyzer (LECO Corporation, St. Joseph, MI). Total starch was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Fat was determined using the Soxhlet extraction procedure (Method 991.36; AOAC, 2005). Calcium and P were determined by colorimetric methods after ashing the samples at 550°C and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005).

Calculations

All data were expressed on a DM basis, and the AME was calculated using the following formula:

$$\text{AME (kcal / kg diet)} = [(\text{FI} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})] / \text{FI}$$

Coefficient of apparent ileal digestibility (**CAID**) of nutrients was calculated using the following formula:

$$\text{CAID of nutrient} = \left[(\text{Nutrient/Ti})_{\text{diet}} - (\text{Nutrient/Ti})_{\text{ileal}}\right] / (\text{Nutrient/Ti})_{\text{diet}}$$

where (Nutrient/Ti)d = ratio of nutrient to Ti in the diet, and (Nutrient/Ti)i = ratio of nutrient to Ti in the ileal digesta.

Statistical Analysis

Cage mean values were considered as the experimental unit, and the data were analyzed in a completely randomized design using the General Linear Models procedure of the SAS Institute Inc. (version 9.4; 2015). A 1-way ANOVA was used in experiment 1 and a 2-way ANOVA in experiment 2 to determine the main effects (acidifier inclusion and CT) and their interaction. Statements of significance were based on P-value of equal to or <0.05, and a P-value between 0.05 and 0.10 was considered as a trend. The Least Significant Difference test was used to separate significant differences between means.

RESULTS

Experiment 1

The phytase recovery from the nonconditioned diet and diets conditioned at 60°C and 90°C were 137, 131, and 126%, respectively. The mortality was minimal (2.1%) and not related to any specific treatment. Conditioning temperature had no effect ($P > 0.05$) on the weight gain (Table 2). Birds fed the diet conditioned at 90°C consumed more ($P < 0.05$) feed than those fed nonconditioned diet but similar ($P > 0.05$) to the diet conditioned at 60°C. There was a tendency ($P = 0.087$) for CT to affect F:G. Birds fed the diet conditioned at 90°C tended to have a higher F:G than those fed the nonconditioned diet.

Steam-conditioning either at 60°C or 90°C reduced ($P < 0.05$) the CAID of DM compared with nonconditioned diet but failed to have any effect on those of fat, Ca, and P (Table 3). A tendency was noted for CT to

Table 2. Influence of conditioning temperature on the weight gain (g/bird), feed intake (g/bird), and feed per gain (g/g) of broiler starters (day 1–21; Exp. 1).[1]

Conditioning temperature, °C	Weight gain	Feed intake	Feed per gain
No conditioning	824	1098[b]	1.334
60	843	1127[a,b]	1.346
90	860	1183[a]	1.386
Probabilities, P ≤	0.346	0.019	0.087
Pooled SEM	16.6	19.0	0.0163

[a-b]Means in a column not sharing a common letter are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate).

affect the CAID of N ($P = 0.071$) and starch ($P = 0.093$). Compared with the nonconditioned diet, the CAID of N and starch were unaffected ($P > 0.05$) by CT at 60°C but tended to reduce when the CT increased to 90°C. Conditioning at 90°C resulted in lower ($P < 0.05$) AME and DM retention compared with nonconditioned diet and the diet conditioned at 60°C.

Experiment 2

The average phytase recovery from the diets conditioned at 60°C and 90°C (regardless of acidifier inclusion) were 101 and 95%, respectively. Influence of FA and CT on the performance parameters of broiler starters fed pelleted diets and PDI is summarized in Table 4. Mortality during the experiment was negligible. Only 6 out of the 288 birds died, and the deaths were not related to any specific treatment. Neither the main effects nor the interaction between acidifier addition and CT was significant ($P > 0.05$) for weight gain, FI, and F:G. However, there was a tendency ($P = 0.066$) for the acidifier to increase the FI at 10.0 g/kg inclusion compared with the diets with no acidifier. While FA and increasing CT improved PDI, the CT effect was attenuated in diets containing acidifier, resulting in a significant acidifier × CT interaction ($P < 0.001$; Table 4).

Influence of FA and CT on the digestibility of nutrients, AME, and retention of DM in broiler starters is summarized in Table 5. The main effect of acidifier inclusion was significant for the CAID of DM ($P < 0.001$), N, starch, P ($P < 0.05$), and fat ($P < 0.01$), with the acidifier increasing the CAID of DM, N, fat, and P at both inclusion levels and of starch at 10 g/kg inclusion. Conditioning at 90°C reduced ($P < 0.05$) the CAID of DM, starch, fat, and Ca, regardless of acidifier inclusion, as indicated by the lack of interaction ($P > 0.05$) between acidifier and CT. Neither the main effects nor the interaction was significant ($P > 0.05$) for AME and DM retention.

DISCUSSION

The paramount motive for this study was to investigate whether i) increasing CT *per se*, in isolation from feed form, have an effect on nutrient utilization and performance of birds (experiment 1) and ii) FA will interact with CT on the nutrient utilization and growth performance of broilers (experiment 2). Predictably, steam-conditioning, either at 60°C or 90°C, reduced the CAID of DM by an average of 5.3% compared with the nonconditioned diet (0.653 vs. 0.689) in experiment 1. Though not significant, birds fed the diet conditioned at 90°C tended to digest N and starch less efficiently by 3.3 and 1.2%, respectively, compared with those fed the nonconditioned diet. Increasing CT to 90°C also lowered AME by 124 kcal/kg and DM retention by 3.2% when compared with the nonconditioned diet. Moreover, conditioning at 90°C increased the FI by 85 g/bird compared with the nonconditioned diet; a finding that can be explained by the AME loss in birds fed the diet conditioned at 90°C. These findings emphasize the fact that high CT impairs nutrient and energy utilization and that feed efficiency is disadvantaged consequently. This is evidenced by deterioration (although only a tendency) of F:G by 5.2 points in birds fed the diet

Table 3. Influence of conditioning temperature on the coefficient of apparent ileal digestibility (CAID)[1] of DM, nitrogen (N), starch, fat, calcium (Ca), phosphorous (P), and AME (kcal/kg DM)[2] and total tract retention (TTR; %)[2] of DM in broiler starters (Exp. 1).

	CAID							
Conditioning temperature, °C	DM	N	Starch	Fat	Ca	P	AME	TTR of DM
No conditioning	0.689[a]	0.837	0.978	0.919	0.510	0.497	3389[a]	72.4[a]
60	0.651[b]	0.822	0.970	0.913	0.478	0.504	3351[a]	71.1[a,b]
90	0.654[b]	0.809	0.966	0.897	0.476	0.474	3265[b]	70.1[b]
Probabilities, P ≤	0.043	0.071	0.093	0.298	0.594	0.577	0.006	0.039
Pooled SEM	0.0106	0.0080	0.0036	0.0098	0.0257	0.0208	24.1	0.566

[a-c]Means in a column not sharing a common letter are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate), measured on day 21.
[2]Each value represents the mean of 6 replicates (8 birds per replicate), measured from day 17 to 21 posthatch.

Table 4. Influence of acidifier addition and conditioning temperature (CT) on the weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) of broiler starters (day 1–21)[1], and pellet durability index (PDI; %; Exp. 2).[2]

Item		Growth performance			Pellet quality
Acidifier (g/kg)	CT, °C	Weight gain	Feed intake	Feed per gain	PDI
0.0	60	1,022	1,351	1.333	75.7[e]
	90	1,029	1,364	1.330	88.7[b]
7.0	60	1,061	1,389	1.310	82.7[d]
	90	1,033	1,370	1.333	88.2[b]
10.0	60	1,049	1,394	1.337	84.2[c]
	90	1,043	1,401	1.344	90.3[a]
Pooled SEM		13.3	16.4	0.0111	0.36
Main effects					
Acidifier					
0.0		1,025	1,358	1.331	82.2
7.0		1,047	1,380	1.321	85.4
10.0		1,046	1,398	1.340	87.2
	CT, °C				
	60	1,044	1,378	1.326	80.8
	90	1,035	1,378	1.336	89.1
Probabilities, P ≤					
Acidifier		0.207	0.066	0.246	0.001
CT		0.422	0.984	0.321	0.001
Acidifier x CT		0.417	0.598	0.531	0.001

[a-e]Means in a column not sharing a common letter are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]Each value represents the mean of 6 replicate samples.

conditioned at 90°C compared with the nonconditioned diet and by 3.0 points compared with the diet conditioned at 60°C in experiment 1. Although similar outcomes for the application of high CT in pelleted diets are not without precedents (Kirkpinar and Basmacioglu, 2006; Abdollahi et al., 2010a,b; Loar II et al., 2014), limited studies to date have investigated the impact of CT in steam-conditioned mash diets. Abdollahi et al. (2011) compared wheat-based mash diets either unconditioned or steam-conditioned at 60°C, 75°C, or 90°C and reported that while introduction of steam at 60°C resulted in slight improvements in N and starch digestibility, increasing CT from 60°C to 75°C and 90°C decreased N digestibility from 0.869 to 0.855 and 0.847 and starch digestibility from 0.977 to 0.940 and 0.913, respectively. In their study, although

Table 5. Influence of acidifier addition and conditioning temperature (CT) on the coefficient of apparent ileal digestibility (CAID)[1] of DM, nitrogen (N), starch, fat, calcium (Ca), phosphorus (P), and AME (kcal/kg DM)[2] and total tract retention (TTR; %)[2] of DM in broiler starters (Exp. 2).

		CAID							
Acidifier (g/kg)	CT, °C	DM	N	Starch	Fat	Ca	P	AME	TTR of DM
0.0	60	0.612	0.783	0.940	0.885	0.215	0.494	3,301	69.9
	90	0.593	0.766	0.936	0.875	0.121	0.474	3,296	70.2
7.0	60	0.663	0.807	0.955	0.920	0.292	0.578	3,317	70.3
	90	0.629	0.789	0.929	0.897	0.168	0.545	3,296	69.4
10.0	60	0.647	0.794	0.957	0.909	0.249	0.561	3,313	70.5
	90	0.632	0.800	0.953	0.890	0.189	0.531	3,286	70.2
Pooled SEM		0.0105	0.0097	0.0061	0.0081	0.0417	0.0265	13.1	0.34
Main effects									
Acidifier									
0.0		0.603[b]	0.774[b]	0.938[b]	0.880[b]	0.168	0.484[b]	3,298	70.1
7.0		0.646[a]	0.798[a]	0.942[b]	0.909[a]	0.230	0.562[a]	3,306	69.8
10.0		0.639[a]	0.797[a]	0.955[a]	0.899[a]	0.219	0.546[a]	3,300	70.3
	CT, °C								
	60	0.641[a]	0.794	0.951[a]	0.905[a]	0.252[a]	0.544	3,310	70.2
	90	0.618[b]	0.785	0.939[b]	0.888[b]	0.160[b]	0.517	3,293	69.9
Probabilities, P ≤									
Acidifier		0.001	0.034	0.025	0.005	0.296	0.015	0.833	0.423
CT		0.013	0.260	0.029	0.015	0.011	0.208	0.117	0.373
Acidifier x CT		0.641	0.373	0.131	0.729	0.744	0.968	0.653	0.242

[a-b]Means in a column not sharing a common letter are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate), measured on day 21.
[2]Each value represents the mean of 6 replicates (8 birds per replicate), measured from day 17 to 21 posthatch.

increasing CT resulted in higher gelatinized starch content (g per kg total starch) from 52.4 in unconditioned mash diet to 82.7 g/kg at 90°C, it also increased resistant starch content by 1.6 g/kg (from 9.5 to 11.1 g per kg total starch); the latter could explain, at least in part, the reduction in starch digestibility of the diets conditioned at higher temperatures. Increasing CT from 60°C to 90°C also reduced the AME of diet by 24 kcal/kg for every 10°C increase in CT (3,317 vs. 3,389). Similar to the current findings, these researchers also reported an increase of 6.6 points in F:G as a result of steam-conditioning at 90°C compared with the unconditioned diet and an increase of 5.5 points compared with the diet conditioned at 60°C.

Despite significant effect of CT on the CAID of some nutrients in experiment 2, it had no impact on growth performance of broilers. It is noteworthy that performance of the birds is not only driven by the digestibility of nutrients. Other factors such as FI and pellet quality, which can influence the energy-sparing effect of pelleted diets, play an important role in determining the growth response and feed efficiency. In the current study, conditioning at 90°C, compared with 60°C, resulted in same FI but supported a higher PDI by 10.3% (89.1 vs. 80.8%), which could have potentially compensated for the deteriorated DM, starch, and fat digestibility in diets conditioned at 90°C. A good pellet quality reduces the heat increment and increases the productive energy of the diet to be used for productive purposes (Latshaw and Moritz, 2009), enabling reduction of dietary energy density with no negative impact on growth performance (McKinney and Teeter, 2004). A contribution of 151 (Skinner-Noble et al., 2005) to 187 (McKinney and Teeter, 2004) kcal MEn/kg diet has been estimated for a diet at 100% pellets compared with the same diet in mash form.

Increasing CT from 60°C to 90°C benefited the PDI, regardless of the acidifier inclusion, but advantage diminished from 17.2% in diets without acidifier (88.7 vs. 75.7%) to 6.6% in diets with 7.0 g/kg acidifier (88.2 vs. 82.7%) and 7.2% in diets with 10 g/kg acidifier (90.3 vs. 84.2%). Comparing only the diets conditioned at 60°C in the current study, the highest PDI was achieved in the diet with 10 g/kg acidifier (84.2%) followed by the diet with 7.0 g/kg acidifier (82.7%), and the diet with no acidifier (75.7%). The acidifier used in the current study was in liquid form with water content of 18 ± 2%. Therefore, the addition of acidifier to the diets at 7.0 and 10 g/kg would have potentially added 1.26 to 1.8 g/kg extra moisture to the diets, respectively. Significant increases in PDI by water addition to corn-based (Moritz et al., 2003), wheat-based (Abdollahi et al., 2012), and barley-based and corn-based diets (Lundblad et al., 2009) have been reported previously. In a conventional pelleting process, the moisture from steam is held largely on the surface of feed particles rather than permeating the starch granules (Smith, 1959). Prepelleting moisture addition probably facilitates moisture permeation to the starch granules, resulting in evenly distributed starch gelatinization throughout the pellet and improves the pellet quality. One implication of this work and previous studies (Abdollahi et al., 2012, 2013b) is that enhancing physical quality of pellets without the application of high CT is a possible strategy.

The current work failed to show any performance benefits with FA, which is in agreement with the results of Gunal et al. (2006), Hernandez et al. (2006), and Houshmand et al. (2012), who did not find any positive effect of FA on growth performance and feed efficiency of broilers. The lack of advantages arising from FA in these studies, and also the present work, could be attributed to the optimal nutrition, hygiene, and management in the experimental condition which did not leave much room for the acidifier to enhance the performance despite improving the digestibility of nutrients (Hernandez et al., 2006; Houshmand et al., 2012).

The present study, however, did confirm the benefits of acidifier in terms of enhanced nutrient digestibility, with FA increasing the CAID of DM, N, starch, fat, and P by an average of 6.6, 3.0, 1.1, 2.7, and 14.5%, respectively. This finding is consistent with study by Palamidi et al. (2017) who reported that dietary acidifier (mixture of formic, propionic, acetic acid, and their salts) supplementation improved the retention of DM, organic matter, and energy by 5.4, 4.8, and 8.8%, respectively. An improved digestibility of DM, protein, and neutral detergent fiber in broilers because of FA by citric acid has also been reported by Ao et al. (2009). Evidence indicates that organic acids induce positive effects on the gut morphology architecture and improve the intestinal absorption capacity (Senkoylu et al., 2007; Adil et al., 2011). According to Lückstädt and Mellor (2011), a lower pH induced by organic acid inclusion might enhance absorption of minerals, which their solubility is pH dependent (Champagne, 1988; Selle et al., 2000) and of proteins through an increase in pepsin activity. However, in a study by Palamidi et al. (2017), gizzard digesta pH was not affected by acidifier addition to a mash diet. Feeding of pelleted diets has been shown to negatively influence the nutrient digestibility (Abdollahi et al., 2013c, 2014), a finding which was partly attributed to an increase in foregut, especially gizzard, pH because of a suboptimal proventriculus and gizzard development induced by feeding textureless pelleted diets (Frikha et al. 2009; Saldana et al. 2015a,b; Naderinejad et al., 2016). Interestingly, in a recent unpublished study from our laboratory, acidification of a wheat-based diet in mash form with same acidifier as the one used in the current study at 7.0 g/kg failed to improve the digestibility of nutrients, suggesting that the beneficial effects of FA might be more pronounced in pelleted diets than those in mash form. Ajandouz and Puigserver (1999), in a study with mixtures of glucose and individual amino acids (Lys, Met, and Thr), reported that the rate of the Maillard "browning" reaction increased with increasing the pH, particularly in the Lys-containing solution. These researchers reported almost no loss of Lys at pH between 4.0 and 6.0 and suggested that the decrease in pH of amino acid–containing glucose mixtures, especially above

neutral pH, can lower the rate of Maillard reactant losses (i.e., sugar and amino acids). This might suggest that the positive effect of FA on digestibility of N and starch in pelleted diets in current study could be due, in part, to the inhibition of Maillard product formation. It is well documented that some of the Maillard specific reactions are irreversible and result in enzymatically undegradable end products, leading to a reduction in availability of nutrients involved in Maillard reaction (Camire et al., 1990).

The lack of significant acidifier × CT interactions observed for the CAID of nutrients in experiment 2 indicated that the effect of CT was similar across both acidifier inclusion rates. However, feeding diets conditioned at 90°C significantly reduced the digestibility of DM by 3.6%, starch by 1.3%, fat by 1.9% and Ca by 36.5%, compared with those conditioned at 60°C. Several studies have shown negative impacts of high CT on nutrient digestibility and bird performance fed pelleted diets (Raastad and Skrede, 2003; Abdollahi et al., 2010a,b, 2011; Loar II et al., 2014). Abdollahi et al. (2011) reported decreases in the CAID of N by 2.2% and starch by 3.9% in a wheat-based diet conditioned at 90°C compared with the diet conditioned at 60°C. Abdollahi et al. (2010a), in a study with wheat-based and corn-based diets, found that increasing the CT from 60°C to 90°C in wheat-based diets deteriorated the CAID of N (0.802 vs. 0.823) and starch (0.898 vs. 0.933), but had no effect in corn-based diets. In a follow-up study (Abdollahi et al., 2010b), conditioning a sorghum-based diet at 90°C impaired N (0.753 vs. 0.776) and starch (0.914 vs. 0.937) digestibility compared with 60°C. However, Loar II et al. (2014), in a study with a corn–soybean meal diet, increased CT from 74°C to 85°C and 96°C and reported a reduction of 3 to 5% in the digestibility of some amino acids which was associated with a loss of feed efficiency by 3 points at 85°C (1.96 vs. 1.99) and by 8 points at 96°C (1.96 vs. 2.04). Several explanations may be provided for the adverse effects of CT on nutrient utilization and bird performance and include i) increased diet/digesta viscosity because of an increase in the solubility of nonstarch polysaccharides (Samarasinghe et al., 2000; Cowieson et al., 2005); ii) inactivation of dietary endogenous and microbial enzymes that contributes to an increase in molecular weight moieties and viscosity (Silversides and Bedford 1999; Cowieson et al., 2005); iii) promotion of excessive and unintentional bacterial growth and toxin production in the small intestine, caused by increased intestinal viscosity, that could potentially compete with the host for nutrients and promote the occurrence of clinical diseases such as necrotic enteritis; iv) retrogradation of starch molecules and formation of resistant starch with negative impact on starch digestibility (Abdollahi et al., 2010b, 2011); v) formation of indigestible linkages such as disulphide bonds in kafirin protein in sorghum (Selle et al., 2010, 2012); and vi) possible degradation of amino acids, especially Cys, Lys, Arg, Thr, and Ser, and reducing their digestibility (Camire et al., 1990; Marsman et al., 1995). While it is tempting to suggest that CT effect on nutrient utilization in poultry diets may vary depending on the type of ingredients used in the diet (Abdollahi et al., 2010a,b), an important implication from the abovementioned studies is that although the conventional CT (generally between 80°C and 90°C) improves the physical pellet quality and feed hygiene, these benefits come at the expense of nutrient digestibility.

CONCLUSIONS

In conclusion, FA, through inclusion of organic acids, is shown to be beneficial to nutrient digestibility in broilers fed pelleted diets. The results of this study also confirm the previously reported detrimental effects of application of high CT in poultry feed manufacture. The current findings may suggest that broiler diets can be conditioned at lower temperatures than the current industry practice if combined with FA. However, a holistic framework investigating the effects of FA and CT on feed hygiene, physical pellet quality, growth performance, nutrient utilization, and intestinal health and histology is required. Such a study will help to unravel the possibility to manufacture hygienic pellets of high quality and high digestibility.

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