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Technical Note

Feeding High-Moisture Corn Grain Silage to Broilers Fed Alternative Diets and Maintained at Different Environmental Temperatures

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ABSTRACT

The effects of the dietary substitution of dry corn by high-moisture corn grain silage (HMCGS) were evaluated on the performance, nutrient digestibility and serum biochemical parameters of broilers reared in an alternative production system and submitted to different environmental temperatures. A total of 288 one-day-old male Cobb chicks were distributed according to a randomized block design in a 3x4 factorial arrangement: three environmental temperatures (hot, thermoneutral or cold) and four levels of HMCGS in substitution of dry corn (0%, 20%, 40% or 60%). The acid analysis showed that the evaluated HMCGS contained average percentage values of ethanol, lactic acid, and acetic acid (expressed in 100% of dry matter) of 0.7690, 2.7320 and 0.0249%, respectively. Propionic and butyric acids were not detected. Dry corn and HMCGS presented pH values of 5.8 and 3.3, respectively. The inclusion of HMCGS reduced dietary pH, as shown by the values of 5.7, 5.4, 5.1 and 4.8 recorded for the diets containing 0%, 20%, 40% and 60% of HMCGS, respectively. There was no significant interaction between diets and environmental temperature. HMCGS may replace up to 40% dry corn in broiler diets when performance, triglyceride levels, and HDL-cholesterol ratio is considered, and up to 60% when nutrient digestibility is evaluated. High environmental temperature impairs broiler performance, nutrient digestibility, and serum biochemistry, demonstrating the influence of environmental temperature on broiler metabolism and performance.

INTRODUCTION

Alternative broiler production presents unique characteristics, because all diets supplied during the rearing period should not include animal products, antibiotics, growth promoters, anticoccidials or any other chemotherapeutic drugs, where as homeopathy and the use of phytotherapeutics are allowed (Demattê Filho & Mendes, 2001). In this kind of production, one of the alternatives to antimicrobial drugs is the dietary addition of organic acids.

The inclusion of high-moisture corn grain silage (HMCGS) in broiler diets as an alternative feedstuff may allow better production indices. High moisture corn silage is a source of organic acids, which have antimicrobial properties, characterizing HMCGS as an ingredient with properties that may potentially replace anticoccidials, antibiotic growth promoters and chemotherapeutics, as required by in alternative broiler production. The inhibition of microbial growth by organic acids is explained by the ability of these acids to cross the microbial membrane and dissociate into the cells, acidifying the cytoplasm (Immerseel *et al*., 2006).

The complete replacement of dry grains by HMCGS in broiler diets was studied by Martins *et al*. (2000), who found reduced live weight at

42 days of age possibly due to the physical limitation of the digestive tract and to high moisture content of the diets containing HMCGS. However, Martinez *et al*. (2001) showed that the inclusion of 100% corn silage in finisher diets does not change performance results. In addition, reports showed that the dietary replacement of dry grains by 50% (Andrade *et al*., 2002) and 60% HMCGS (Gonçalves *et al*., 2005) did not affect the performance of broilers reared until 49 days. Barcellos *et al*. (2006) found that replacing 100% corn by wet grain silage of low-tannin sorghum in broiler diets did not impair their performance and reduced the cost per pound produced.

All those benefits may vary according to the level of dry corn substitution by HMCGS (Sartori *et al*., 2002; Gonçalves *et al*., 2007), as well as to the environmental conditions to which broilers are exposed. The average environmental temperature considered comfortable for broilers is around 26°C. At this temperature, air relative humidity and velocity have little influence on broiler performance and its physiology. They remain quiet, do not shiver, are uniformly distributed in the shed, and present adequate feed intake, weight gain and excellent feed conversion ratio (Medeiros *et al.,* 2005).

Gomes & Macari (2000) stated that tropical and subtropical countries present high potential for poultry production, but one of the main obstacles for its progress is the hot weather. Daytime temperatures exceeding 30° to 32°C are considered stressful for broilers and have a negative effect on their performance, as they reduce their feed intake.

When kept under heat stress, broilers reduce their growth rate and feed intake (Oliveira *et al*., 2006a; Oliveira *et al*., 2006b), resulting in worse feed conversion ratio (Yahav, 1999; Sartori *et al*., 2001; Sahin *et al*., 2003). Under these conditions, broilers increase their respiratory rate in order to stimulate evaporative heat loss (panting) and to maintain body heat balance, which may affect their metabolism (Silva *et al*., 2001; Yahav *et al*., 2005).

In regions with climates with high daily temperatures, poultry production losses are potentially high, because they include both direct and indirect losses (Salgado & Nääs, 2010). Therefore, the thermal environment is considered a major stressor in poultry.

The objective of the present study was to evaluate the effects of different HMCGS dietary inclusion levels in substitution of dry corn on the performance, nutrient digestibility and serum triglyceride and cholesterol levels of broilers reared in an alternative system an submitted to different environmental temperatures.

MATERIAL AND METHODS

Birds and Treatments

Two hundred eighty-eight one-day-old male Cobb chicks were housed in 48 galvanized wire cages (0.50 m high, 0.50 m wide and 0.60 m deep) equipped with an individual feeder and nipple drinkers, with six chickens per cage, distributed in three environmentally-controlled chambers (hot, thermoneutral, and cold environments). The cages were laid out in two batteries with two tiers each, totaling 16 cages per chamber.

All broilers remained in the hot chamber for up to seven days old in order not to affect their initial performance. On day eight, they were randomly distributed to the chambers according to a randomized block design in a 3x4 factorial arrangement, consisting of three environmental temperatures (hot, thermoneutral or cold) and four diets (T_0, T_{20}, T_{40}, T_{60}, corresponding to the inclusion of 0%, 20%, 40% and 60% of high-moisture corn grain silage -HMCGS - in substitution to dry corn, respectively). There were four replications of six birds per experimental unit in each treatment.

The chambers were equipped with a temperature and moisture control system and exhaustion fan to allow for air renovation and gas elimination. Heating and/or cooling of the three chambers was monitored by an automatic temperature and moisture control system (Table 1), through electronic sensors connected to the computerized program *Master Plant* especially developed for this purpose, which continually recorded temperature and moisture values. Time intervals for air renovation in the chambers were programmed by timers in the general control panel of the chamber to reduce ammonia accumulation. Lighting was continuously provided by 40-watt fluorescent bulbs.

Table 1 – Environmental temperatures applied during experimental period.

Age (days)	Environmental Temperature (°C)		
	Thermoneutral	Hot	Cold
1-7	-	35	-
8-10	30	34	21
11-14	29	34	21
14-16	28	34	19
16-21	26	33	17
22-49	24	33	15

Diets

Semi-hard texture hybrid Co32 (Dow AgroSciences Company) corn, containing 26.57% moisture was used to prepared the HMCGS. After mechanical harvest, corn was ground (4mm particle size) and compacted in 220kg plastic drums, according the method described by Costa *et al.* (1999) and Nummer Filho (2001). The same corn hybrid was used for manufacturing the feed with dry grains (12% moisture), and ground to the same particle size used to process high-moisture corn.

The HMCGS drums were opened after two months of fermentation. The top layer of approximately 10 cm thickness was discarded in each drum, due to possible growth of microorganisms on the surface.

After the 2-month storage period, the HMCGS used in this experiment was evaluated in a digestibility assay, and presented gross energy (GE) and apparent metabolizable energy corrected for nitrogen (AMEn) levels of 4,842.26 kcal GE/kg and 3,496.65 kcal AMEn/kg, respectively.

The feeding program was divided into three phases: starter- 1 to 21 days (Table 2), grower- 22 to 42 days (Table 3) and finisher- 43 to 49 days (Table 4), according to the recommendations of Rostagno *et al*. (2000). Feeds contained equal crude protein and AMEn levels.

Table 2 – Ingredients and calculated nutritional composition of the starter experimental diets (1 to 21 days).

Ingredients (%)	Starter			
	T0	T20	T40	T60
Corn	58.389	49.140	42.410	37.294
HMCGS[1]	-	9.828	16.964	22.376
Soybean meal	35.450	35.166	34.960	34.819
Dicalcium phosphate	1.820	1.820	1.820	1.820
Limestone	1.000	0.990	0.990	0.990
DL-Methionine	0.230	0.235	0.235	0.235
L-Lysine	0.170	0.180	0.180	0.185
Choline chloride (70%)	0.041	0.041	0.041	0.041
Salt	0.350	0.350	0.350	0.350
Soybean oil	2.400	2.100	1.900	1.740
Vitamin mix[2]	0.100	0.100	0.100	0.100
Mineral mix[3]	0.050	0.050	0.050	0.050
Calculated composition				
AMEn, kcal/kg	3000	3000	3000	3000
Crude Protein, %	21.40	21.40	21.40	21.40
Calcium, %	0.97	0.96	0.96	0.96
Available phosphorus, %	0.45	0.45	0.45	0.45
Crude fiber, %	3.24	3.29	3.32	3.35
Methionine, %	0.56	0.56	0.56	0.56
Methionine + cystine, %	0.89	0.90	0.90	0.90
Lysine, %	1.27	1.27	1.26	1.26
Potassium, %	0.83	0.82	0.82	0.82
Sodium, %	0.18	0.18	0.18	0.18
Chlorine, %	0.24	0.24	0.24	0.24
Linoleic acid, %	2.67	2.51	2.41	2.33

[1]HMCGS - High-moisture corn grain silage.
[2]Vaccinar Animal Nutrition. Vitamin mix (per kg): retinyl acetate 2,580 mg, cholecalciferol 38.4 mg, α-tocopherol 60 mg, menadione 8 mg, vitamin B_1 6 mg, vitamin B_2 12 mg, vitamin B_6 12 mg, cyanocobalamin 60 µg, niacin 80 mg, pantothenic acid 30 mg, biotin 0.240 mg, folic acid 3 mg, vitamin C 100 mg, antioxidant 0.125 mg.
[3]Vaccinar Animal Nutrition. Mineral mix (per kg): selenium 0.72 mg, iodine 2.80 mg, iron 192 mg, copper 40 mg, manganese 312 mg, zinc 220 mg.

Table 3 – Ingredients and calculated nutritional composition of the grower experimental diets (22 to 42 days).

Ingredients (%)	Growth			
	T0	T20	T40	T60
Corn	63.757	53.590	46.200	40.625
HMCGS[1]	-	10.718	18.480	24.375
Soybean meal	29.800	29.524	29.322	29.170
Dicalcium phosphate	1.620	1.620	1.620	1.620
Limestone	0.940	0.950	0.975	0.975
DL-Methionine	0.160	0.175	0.180	0.180
L-Lysine	0.220	0.220	0.230	0.232
Choline chloride (70%)	0.053	0.053	0.053	0.053
Salt	0.350	0.350	0.350	0.350
Soybean oil	2.950	2.650	2.440	2.270
Vitamin mix[2]	0.100	0.100	0.100	0.100
Mineral mix[3]	0.050	0.050	0.050	0.050
Calculated composition				
AMEn, kcal/kg	3100	3100	3100	3100
Crude Protein, %	19.30	19.30	19.30	19.30
Calcium, %	0.88	0.88	0.89	0.89
Available phosphorus, %	0.41	0.41	0.41	0.41
Crude fiber, %	3.01	3.06	3.10	3.13
Methionine, %	0.46	0.47	0.48	0.48
Methionine + cystine, %	0.77	0.79	0.79	0.79
Lysine, %	1.16	1.16	1.16	1.16
Potassium, %	0.74	0.74	0.73	0.73
Sodium, %	0.18	0.18	0.18	0.18
Chlorine, %	0.24	0.24	0.24	0.24
Linoleic acid, %	3.03	2.87	2.76	2.68

[1]HMCGS - High-moisture corn grain silage.
[2]Vaccinar Animal Nutrition. Vitamin mix (per kg): retinyl acetate 860 mg, cholecalciferol 12.8 mg, α-tocopherol 20 mg, menadione 2 mg, vitamin B_1 2 mg, vitamin B_2 4 mg, vitamin B_6 4 mg, cyanocobalamin 20 µg, niacin 30 mg, pantothenic acid 10 mg, biotin 0.060 mg, folic acid 1 mg, vitamin C 50 mg, antioxidant 0.125 mg.
[3]Vaccinar Animal Nutrition. Mineral mix (per kg): selenium 0.72 mg, iodine 2.80 mg, iron 192 mg, copper 40 mg, manganese 312 mg, zinc 220 mg.

Silage was included adopting the correction factor of 1.198 obtained by the ratio between dry corn (88.00%) and silage (73.43%) dry matter contents in order to obtain equal dry matter content in all diets.

Diet ingredients, except HMCGS, had been previously mixed, composing the so-called nucleus. HMCGS was incorporated to the diets on a daily. Water and feed were supplied *ad libitum*. Daily, residues of feeds containing HMCGS were collected, weighed and discarded.

Sample Collection and Analysis

Feed, dry corn and silage samples were submitted to pH analysis. Twenty grams of each sample were mixed in 30mL deionized water until homogenization, stirred using a magnetic bar and electric mixer for 10 minutes, and pH was immediately read using a pH meter. Each sample was read in triplicate and their average was taken as pH value (Lopes *et al*., 2002).

Table 4 – Ingredients and calculated nutritional composition of the finisher experimental diets (43 to 49 days).

Ingredients (%)	Final			
	T0	T20	T40	T60
Corn	66.288	55.701	48.068	42.260
HMCGS[1]	-	11.140	19.227	25.356
Soybean meal	26.550	26.300	26.071	25.921
Dicalcium phosphate	1.430	1.420	1.420	1.420
Limestone	0.890	0.900	0.900	0.900
DL-Methionine	0.160	0.159	0.160	0.160
L-Lysine	0.170	0.178	0.187	0.190
Choline chloride (70%)	0.037	0.037	0.037	0.037
Salt	0.350	0.350	0.350	0.350
Soybean oil	3.975	3.665	3.430	3.256
Vitamin mix[2]	0.100	0.100	0.100	0.100
Mineral mix[3]	0.050	0.050	0.050	0.050
Calculated composition				
AMEn, kcal/kg	3200	3200	3200	3200
Crude Protein, %	18.00	18.00	18.00	18.00
Calcium, %	0.80	0.80	0.80	0.80
Available phosphorus, %	0.37	0.37	0.37	0.37
Crude fiber, %	2.86	2.92	2.96	2.99
Methionine, %	0.44	0.44	0.44	0.44
Methionine + cystine, %	0.74	0.74	0.74	0.74
Lysine, %	1.04	1.04	1.04	1.04
Potassium, %	0.69	0.68	0.68	0.68
Sodium, %	0.18	0.18	0.18	0.18
Chlorine, %	0.24	0.24	0.24	0.25
Linoleic acid, %	3.60	3.45	3.33	3.24

[1]HMCGS - High-moisture corn grain silage.
[2]Vaccinar Animal Nutrition. Vitamin mix (per kg): retinyl acetate 688 mg, cholecalciferol 9.6 mg, α-tocopherol 15 mg, menadione 2 mg, vitamin B_1 1 mg, vitamin B_2 3 mg, vitamin B_6 2 mg, cyanocobalamin 15 µg, niacin 20 mg, pantothenic acid 8 mg, biotin 0.04 mg, folic acid 0.50 mg, vitamin C 50 mg, antioxidant 0.125 mg.
[3]Vaccinar Animal Nutrition. Mineral mix (per kg): selenium 0.72 mg, iodine 2.80 mg, iron 192 mg, copper 40 mg, manganese 312 mg, zinc 220 mg.

Organic acid analyses were also conducted by collecting of high-moisture grain silage samples, which were processed according to Supelco Bulletin (1998). Lactic acid was determined by liquid chromatography according to Danner *et al*. (2000) and Molnár-Perl (2000). Acetic, butyric, and propionic acid contents and ethanol percentage were determined by gas chromatography (Wilson, 1971).

Performance data were obtained for accumulated periods of 8 to 21 and 8 to 49 days of age. Body weight (BW), weight gain (WG), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR) and mortality were determined.

In order to determine digestibility, excreta samples were collected from all cages for three consecutive days, at12-hour intervals, beginning on day 47 of the experiment, and pooled per replicate. Each excreta pool was placed in an individual plastic bag and stored at -20°C. At the end of the collection period, samples were thawed at room temperature and pre-dried at 55°C in a forced-ventilation oven for 72 hours. After pre-drying, excreta samples were ground and stored for subsequent laboratory analyses. Dry matter (DM), crude protein (CP), crude fiber (CF) and ether extract (EE) contents were determined according to the Weende scheme, following the recommendations of the Association of Official Analytical Chemists (AOAC, 1990).

At 49 days, four broilers per treatment were sacrificed and 5 mL of blood were collected from their jugular veins. Blood was placed into previously identified tubes and centrifuged at 2.000 G for 10 minutes. The extracted serum was stored in a freezer at -20°C. Cholesterol, triglycerides and HDL cholesterol levels were determined by the colorimetric enzymatic method using a commercial kit with readings at 505 nm in spectrophotometer, following Lumeij's (1997) methodology.

Statistical Analysis

Data were submitted to analysis of variance (ANOVA) using SAEG software (1997). The effect of diets, when significant, was split into a polynomial regression analysis. Tukey's test was applied to verify possible the differences among environmental temperatures means. Results were considered significant at 5% significance level.

RESULTS

Dry corn and HMCGS samples presented pH values of 5.8 and 3.3, respectively (Table 5). Diets containing 0%, 20%, 40%, and 60% HMCGS in substitution of dry corn presented pH values of 5.7, 5.4, 5.1 and 4.8, respectively. The HMCGS acid analysis only showed mean percent values for ethanol, lactic acid, and acetic

Table 5 – High-moisture corn grain silage (HMCGS) pH and organic acids profile, expressed in 100% of dry matter, and pH values of dry corn and experimental diets.

	pH	Ethanol (%)	Lactic acid (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
HMCGS	3.3	0.7690	2.7320	0.0249	0.0000	0.0000
				pH		
Dry corn				5.8		
T0[1]				5.7		
T20				5.4		
T40				5.1		
T60				4.8		

[1] T0 - Inclusion of 0% of HMCGS in substitution of dry corn, T20 - Inclusion of 20% of HMCGS in substitution of dry corn, T40 - Inclusion of 40% of HMCGS in substitution of dry corn, T60 - Inclusion of 60% of HMCGS in substitution of dry corn.

acid (expressed in 100% of dry matter) of 0.7690, 2.7320 and 0.0249%, respectively. Propionic and butyric acids were not detected.

There was no significant interaction between diets and environmental temperature for all studied variables. Silage inclusion influenced FCR in both rearing periods (Table 6). Regression analysis showed a quadratic and linear increase in FCR in the periods of 8 to 21 days and of 8 to 49 days, respectively, when dry corn was replaced by HMCGS. The best inclusion of HMCGS level was estimated at 29.17% for FCR at 21 days. The treatments did not affect BW, WG, DWG, FI and mortality in both periods.

Silage inclusion influenced the crude protein (CPD) and ether extract (EED) digestibility (Table 7). Linear increase and quadratic effect were observed for CPD and for EED, respectively; indicating that broilers fed diets with higher HMCGS levels presented better CPD. The EED increased up to 47.96% of HMCGS inclusion, decreasing thereafter.

Silage inclusion affected all blood variables studied (Table 8). Serum cholesterol levels linearly increased as HMCGS inclusion levels increased in the diets. Triglyceride levels showed a quadratic effect, suggesting an inclusion level of 26.85% HMCGS for the lowest TG level. An increase in HDL-cholesterol ("good" cholesterol) levels was observed when up to 37.20% of HMCGS was included in substitution of dry corn.

Between 8 and 21 days (Table 6), broilers kept in the cold environment presented higher BW, WG, and DWG compared with those kept in the thermoneutral environment. However, these results were not significantly different between birds submitted to thermal challenge by heat or cold. During this phase, the FI of broilers maintained in the cold environment was higher than that of other groups, resulting in worse FCR. No mortality was recorded in any of the treatments groups between days 8 and 21. In the period of 8-49 days, broilers maintained in the hot environment presented lower BW, WG, DWG, FI, worse FCR, and higher mortality compared with those kept in the thermoneutral and cold environments, which did were not different from each other. However, FI was higher in the cold environment, decreasing as temperature increased.

Temperature influenced only dry matter (DMD) and crude fiber (CFD) digestibility (Table 7). Broilers kept in thermoneutral temperature showed better results when compared with those kept in the hot and cold environments, which did not differ. No CPD or EED differences were detected among the different environments.

No significant temperature effect was observed for serum cholesterol values (Table 8). Broilers submitted to heat challenge presented higher triglyceride levels than those kept in the cold chamber, which showed

Table 6 – Performance from 8 to 21 days and 8 to 49 days of broilers reared in an alternative production system, as a function of high-moisture corn grain silage (HMCGS) inclusion level in substitution of dry corn and environmental temperature.

Variables[1]	HMCGS levels (%)				Temperature			CV[2]
	0	20	40	60	H	T	C	
				8-21 days				
BW (g) [4]	659	669	671	663	655 [ab]	649 [b]	692 [a]	6.90
WG (g) [4]	531	541	543	536	527 [ab]	522 [b]	565 [a]	8.52
DWG (g) [4]	37.94	38.66	38.79	38.27	37.65 [ab]	37.25 [b]	40.35 [a]	8.52
FI (g) [3, 4]	938	895	923	941	888 [b]	874 [b]	1,010 [a]	6.73
FCR[3, 5]	1.77	1.65	1.70	1.76	1.69 [b]	1.68 [b]	1.79 [a]	4.38
				8-49 days				
BW (g) [4]	2,137	2,296	2,164	2,222	1,653 [b]	2,390 [a]	2,572 [a]	11.70
WG (g) [4]	2,009	2,169	2,037	2,095	1,525 [b]	2,262 [a]	2,445 [a]	12.42
DWG (g) [4]	47.83	51.63	48.50	49.88	36.32 [b]	53.85 [a]	58.20 [a]	12.42
FI (g) [3, 4]	4,119	4,199	4,190	4,410	3,251 [c]	4,424 [b]	5,014 [a]	9.57
FCR[3, 6]	2.10	2.06	2.15	2.21	2.22 [a]	2.04 [b]	2.12 [b]	5.84
MORT [4,7]	2.15	3.54	2.82	2.69	4.82 [b]	1.79 [a]	1.79 [a]	79.37

[1] BW - body weight, WG - weight gain, DWG - daily weight gain, FI - feed intake, FCR - feed conversion ratio, MORT - mortality.
[2] Coefficient of variation (%).
[3] Values corrected on dry corn dry matter basis (88%).
[4] No significant effect of HMCGS levels (P>0.05).
[5] $Y = 0.000105823x^2 - 0.0061731x + 1.75725$ ($R^2 = 0.86$).
[6] $Y = 0.042x + 2.025$ ($R^2 = 0.68$).
[7] Mortality corrected to $(x + 0.5)^{1/2}$, expressed in %.
[a, b, c] Means within the same row with different superscripts differ significantly by Tukey Test (p<0.05).

Table 7 – Nutrient digestibility (%) determined at 49 days of age, as a function of high-moisture corn grain silage (HMCGS) inclusion level in substitution of dry corn and environmental temperature.

Variables [1]	HMCGS Levels (%)				Temperature			CV^2
	0	20	40	60	H	T	C	
DMD [3]	81.31	82.33	81.67	83.37	81.73 ^{b}	83.28 ^{a}	81.50 ^{b}	2.59
CPD [4]	63.09	67.88	67.15	70.24	67.50 ^{ns}	68.51	65.26	6.65
CFD [3]	33.25	33.41	31.88	37.19	33.58 ^{b}	37.10 ^{a}	31.12 ^{b}	9.95
EED [5]	94.76	96.07	96.16	96.37	95.87 ^{ns}	96.08	95.58	0.80

[1] DMD - Dry matter digestibility, CPD - Crude protein digestibility, CFD - Crude fiber digestibility, EED - Ether extract digestibility.
[2] Coefficient of variation (%).
[3] No significant effect of HMCGS levels (P>0.05).
[4] $Y = 2.072x + 61.91$ ($R^2 = 0.80$).
[5] $Y = - 0.000679448x^2 + 0.0651786x + 94.8422$ ($R^2 = 0.94$).
[a, b] Means within the same row with different superscripts differ significantly by Tukey's Test (p<0.05).
[ns] No significant effect (P>0.05).

Table 8 – Serum biochemical parameters (mg/dL) determined at 49 days of age, as a function of high-moisture corn grain silage (HMCGS) inclusion level in substitution of dry corn and environmental temperature.

Variables	HMCGS Levels (%)				Temperature			CV^4
	0	20	40	60	H	T	C	
Chol (mg/dL)[1]	130.36	134.77	155.68	151.69	148.05 ^{ns}	143.26	138.07	9.37
Trig (mg/dL)[2]	57.51	38.11	40.04	66.42	57.91 ^{a}	50.00 ^{ab}	43.65 ^{b}	25.28
HDL (mg/dL)[3]	70.16	81.21	86.12	79.09	73.08 ^{b}	86.34 ^{a}	78.02 ^{b}	11.75

[1] Cholesterol → $Y = 8.49x + 121.9$ ($R^2 = 0.78$).
[2] Triglycerides → $Y = 0.0273172x^2 – 1.46701x + 55.88$ ($R^2 = 1.00$).
[3] HDL - High density lipoprotein → $Y = - 0.0103847x^2 + 0.772581x + 70.2293$ ($R^2 = 0.99$).
[4] Coefficient of variation (%).
[a, b] Means within the same row with different superscripts differ significantly by Tukey'sTest (p<0.05).
[ns] No significant effect (p>0.05).

better results. Under thermoneutral conditions, broilers obtained better HDL cholesterol levels, differently from those kept under heat or cold temperatures.

DISCUSSION

In the present study, since thermal challenge by cold is more aggressive than by heat during the starter phase, broilers consumed more feed in that environment. However, this was not reflected in higher WG at this stage, suggesting that birds used the feed to produce endogenous heat to help them maintaining their body temperature, consequently presenting worse FCR.

Studies show that a high environmental temperature negatively influences WG and FI in broilers reared up to 21 days (Cella *et al*., 2001; Oliveira *et al*., 2006a). Some studies reported that broilers reared in a hot environment between one and 21 days old had better FCR when compared with those kept in a thermoneutral environment (Baziz *et al*., 1990; Zanusso, 1998; Cella *et al*., 2001), differently from the findings of the present study.

Between 8 and 49 days of age, the hot environment was more harmful to broilers than the cold environment, as shown by the worse FCR obtained in the broilers reared in that environment. Additionally, there was compensatory gain in the cold environment after 21 days and that the broilers in the hot environment did not eat enough, which harmed their performance. Similar results were obtained by Lana *et al*. (2000), who found that heat stress negatively influenced FI and, consequently, directly affected WG and FCR of 42-day-old broilers. Oliveira Neto *et al*. (2000) also found that room temperature influenced WG, which was 16% lower for broilers under heat stress relative to those kept under thermal comfort. Similarly to WG, those authors also observed that high temperature (32°C) determined a 19% increase in FCR.

Environmental variables can have both positive and negative effects on broiler production. High temperatures reduce feed intake, which harms broiler performance, whereas low temperatures can improve weight gain, but negatively affect FCR. Oliveira *et al*. (2006b) reported that low temperatures negatively influenced broiler FCR between 22 and 42 days old, and the gradual increase of temperature up to 26.3°C improved it.

Environmental conditions must be controlled as much as possible to prevent negative effects on broiler performance, as they may affect metabolism (body heat production at low temperatures and body heat dissipation at high temperatures), consequently affecting animal production (meat and eggs) and the incidence of metabolic illnesses such as pulmonary hypertension syndrome (ascitis) (Furlan, 2004).

In both rearing periods, silage levels did not affect FI, and consequently BW. However, the best FCR was obtained when 40% of HMCGS was included in substitution by dry corn, showing that higher inclusion levels may negatively influence FCR. Martins *et al*. (2000) found that the total substitution of dry corn by HMCGS in broiler diets reduced live weight at 42 days of age, possibly due to the physical limitation of the digestive tract and a high-moisture content of the diets. However, Martinez *et al*. (2001) showed that 100% silage can be included in broiler diets with no effects on performance, since it provided in the finisher phase (29 to 42 days). Sartori *et al*. (2002) reported that HMCGS could fully replace dry corn in broilers diets up to 21 days of age. Recent research has shown that the use of diets with 50% (Andrade *et al.,* 2002) and 60% HMCGS (Gonçalves *et al*., 2005) in substitution of dry grains does not affect broiler performance up to 49 days.

In our study, the thermoneutral environment allowed better nutrient digestion. Garcia *et al*. (2004), studying corn and sorghum digestibility in broilers maintained at different temperatures, observed that the digestibility of dry matter, protein, fiber and fat was better in the hot environment and worse in the cold one. According to Gomes & Macari (2000), under heat stress, FI decreases, and broilers are less active. They drink two to three times more water, increasing water excretion via panting and urine, resulting in better nutrient digestibility. On the other hand, Bonnet *et al*. (1997) found lower protein and fat digestibility in broilers reared at high environmental temperature.

High HMCGS levels in substitution of dry corn may positively influence CPD and EED. The better digestibility of these nutrients obtained in broilers fed higher HMCGS levels may be attributed to the presence of organic acids, which reduce the competition of the normal microflora with pathogens, endogenous nitrogen losses, and the production of ammonia production and of other growth-depressing factors. Organic acids also reduce diet pH, consequently increasing pepsin activity (Dibner & Buttin, 2002). Furthermore, fatty acids, which are part of ether extract and are detected by laboratory analysis, may improve fat digestibility. The joint action of low pH and high pepsin proteolytic activity agglutinate lipids in feeds (Moran Júnior, 1994). All these factors contribute to improve the digestibility of diets and the viability of alternative poultry production, which feeds do not contain antibiotic growth promoters.

In the present study, thermal environment did not affect serum cholesterol levels, but lower triglyceride levels were detected in broilers reared in the cold environment. Under heat stress and reduced FI, a larger quantity of triglycerides is deposited in the liver, because fats, rather than carbohydrates, are used as energy source. In other words, under these circumstances, large amounts of triglycerides in the adipose tissue are mobilized, transported as free fatty acids in the blood, and later deposited again as triglycerides in the liver, where they are oxidized (Dias, 2004). This is the main cause of high triglyceride levels obtained in the broilers kept in the hot environment in the present study.

The highest HDL to cholesterol ratios were obtained in broilers kept under thermoneutral conditions, indicating that their lipid metabolism was normal, causing HDL to naturally transport cholesterol from peripheral tissues to the liver (Marzzoco & Torres, 1999), which does not happen in thermal stress situations.

Meluzzi *et al*. (1992) determined total cholesterol, triglycerides and HDL-cholesterol reference values of 140.0, 69.8 and 88.8, respectively, for 45-day-old broilers, which is consistent with the data obtained in this experiment. However, Lopes (1994), studying the effect of feed restriction during the winter and the summer on serum biochemical parameters of 49-day-old broilers, observed higher cholesterol and triglyceride levels in the winter than in the summer.

In the present experiment, it was observed that high levels of HMCGS inclusion negatively influenced serum cholesterol levels. Literature is rather scarce regarding HMCGS and its effects on the serum biochemical parameters evaluated. Therefore, research on the possible action of organic acids produced by anaerobic fermentation of silage as natural growth promoter sand their effects on cholesterol, triglycerides, and HDL-cholesterol levels is needed.

The analysis of HMCGS acids showed that their levels are consistent with those considered ideal to ensure good silage after silo opening, which, according to Mahanna (1994), should be between 1.0% and 3.0% for lactic acid; lower than 0.1% for acetic acid; 0 and 1.0% for propionic acid and lower than 0.1% for butyric acid. Gonçalves *et al*. (2005) determined concentrations of 0.4380% ethanol, 3.3130% lactic acid, and 0.0362% acetic acid in HMGCS. Propionic and butyric acids were not detected in the HMCGS evaluated in the present study. Johnson *et al*. (2003), in an experiment with HMCGS made with two corn varieties (flint or dent), observed lactic acid values of 2.87 and 3.41%, respectively. High lactic acid concentration indicates better silage quality, whereas the detection of high butyric acid levels indicates significant dry matter loss, reduction in silage acceptability by the animals, and silage stability (Lopes, 2004).

The inclusion of silage in substitution to dry corn reduced dietary pH. This analysis was also performed by Gonçalves *et al*. (2005), who found pH values of 6.3, 4.0, 6.6, 6.1 and 5.6 for dry corn, HMCGS, and diets with 0%, 30%, and 60% HMCGS inclusion in substitution of dry corn, respectively. Upon studying HMCGS of two corn varieties (flint and dent), Johnson *et al*. (2003) observed pH of 4.05 and 3.86, respectively, after 57 days of storage. Low diet pH due to the presence of organic acids in silage is directly related to better diet digestibility, as it increases starch digestion, which is the main component of grains (Jobim & Reis, 2001).

CONCLUSION

Under the conditions of the present study, it was not possible to observe interactions between the evaluated diets and evaluated temperatures. Considering the performance, serum triglyceride levels, and HDL-cholesterol ratio results, high-moisture corn grain silage may replace up to 40% dry corn in broiler diets, and up to 60% when nutrient digestibility is considered. However, increasing silage levels in the diet of broilers reared in alternative system negatively influences serum cholresterol.

Hot environmental temperatures impair broiler performance, nutrient digestibility, triglyceride levels, and HDL-cholesterol ratios, demonstrating the influence of the thermal environment on broiler metabolism and performance.

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REFERENCES

Andrade RC, Sartori JR, Martinez KLA, Costa C, Pezzato AC, Oliveira HN. Silagem de grãos úmidos de milho e simbiótico na alimentação de frangos de corte criados nos sistemas convencional e alternativo. Revista Brasileira de Ciência Avícola 2002;4:26.

Association of Official Analytical Chemists. Official Methods of Analysis. 15th ed. Washington: DC; 1990.

Barcellos LCG, Furlan AC, Murakami AE, Silva MAA, Silva RM. Avaliação nutricional da silagem de grãos úmidos de sorgo de alto ou de baixo conteúdo de tanino para frangos de corte. Revista Brasileira de Zootecnia 2006;35(1):104-112.

Baziz HA, Geraert PA, Guillaumin S. Effects of high temperature and dietary composition on growth, body and composition energy retention in broilers. Proceedings of the 7th European Poultry Conference; 1990; Barcelona. Espanha. p. 626-629.

Bonnet S, Geraert PA, Lessire M, Carre B, Guillaumin S. Effect of high ambient temperature on feed digestibility in broiler. Poultry Science 1997;76:857-863.

Cella PS, Donzele JL, Oliveira RFM, Albino LFT, Ferreira AS, Gomes PC, Valerio SR, Apolônio LR.Níveis de lisina mantendo a relação aminoacídica para frangos de corte no período de 1 a 21 dias de idade, em diferentes ambientes térmicos.Revista Brasileira de Zootecnia 2001;30(2):433-439.

Costa C, Arrigoni MB, Silveira AC, Chardulo LAL. Silagem de grãos úmidos. Anais do 7º Simpósio sobre Nutrição de Bovinos; 1999; Piracicaba, São Paulo. Brasil. p. 69-87.

Danner H, Madzingaidzo L, Holzer M. Extraction and purification of lactic acid from silages. Bioresource Technology 2000;75(3):181-187.

Demattê Filho LC, Mendes CMI. Viabilidade técnica e econômica na criação alternativa de frangos. Anais da Conferência Apinco de Ciência e Tecnologia Avícolas; 2001; Campinas, São Paulo. Brasil. p. 255-266.

Dias LTS. Efeitos do tanino e do ácido tânico sobre os lipídios plasmáticos e morfometria do fígado e pâncreas em frangos de corte [tese]. Jaboticabal (SP): Universidade Estadual Paulista; 2004.

Dibner JJ, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research 2002;11(4):453-463.

Furlan RL. Gerenciando o desafio da produção de frangos de corte em altas temperaturas sob o enfoque da ambiência [apostila]. Jaboticabal (SP): Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista; 2004. 40p.

Garcia RG, Mendes AA, Sartori JR, Paz ICLA, Takahashi SE, Pelícia K, Komiyama CM, Quinteiro RR.Digestibility of feeds containing sorghum, with and without tannin, for broiler chickens submitted to three room temperatures. Brazilian Journal of Poultry Science 2004;6(1):55-60.

Gomes LFF, Macari M. Efeito do uso de enzimas sobre a digestibilidade de dieta a base de milho e farelo de trigo sob estresse térmico em frangos de corte colostomizados. Revista Brasileira de Ciência Avícola 2000;2:30.

Gonçalves JC, Sartori JR, Pezzato AC, Costa C, Martines KLA, Cruz VC, Madeira LA, Oliveira HN. Silagem de grãos úmidos de milho em substituição ao milho seco da ração de frangos de corte criados em dois sistemas. Pesquisa Agropecuária Brasileira 2005;40(10):1021-1028.

Gonçalves JC, Sartori JR, Cruz VC, Pinheiro DF, Pelícia VC, Costa C. Desempenho e digestibilidade de nutrientes em frangos de corte alternativos alimentados com silagem de grãos úmidos de sorgo. Acta Scientiarum Animal Science 2007;29(3):283-290.

Immerseel FV, Russel JB, Flythe MD, Gantois I, Timbermont L, Pasmans F, Haesebrouck F, Ducatelle R. The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathology 2006;35(3):182-188.

Jobim CC, Reis RA. Produção e utilização de silagem de grãos úmidos de milho. A produção animal na visão dos brasileiros. Anais da 38ª

Reunião Anual da Sociedade Brasileira de Zootecnia; 2001; Piracicaba, São Paulo. Brasil. p. 912-927.

Johnson LM, Harrison JH, Davidson D, Mahanna WC, Shinners K. Corn silage management: Effects of hybrid, maturity, inoculation and mechanical processing on fermentation characteristics. Journal of Dairy Science 2003;86(1):287-308.

Lana GRQ, Rostagno HS, Albino LFT,Lana AMQ. Efeito da temperatura ambiente e da restrição alimentar sobre o desempenho e a composição da carcaça de frangos de corte.Revista Brasileira de Zootecnia 2000;29(4):1117-1123.

Lopes AM. Efeito da restrição alimentar sobre a qualidade da carcaça de frangos de corte [dissertação]. Botucatu (SP): Universidade Estadual Paulista; 1994.

Lopes ABRC, Leonel M, Cereda MP. The effect of the ensilage process of moist corn grains on the microscopic characteristics of the starch. Brazilian Journal of Food Technology 2002;5:177-181.

Lopes ABRC. Efeito de métodos de preservação dos grãos úmidos de milho e de sorgo sobre a estrutura do endosperma, dos grânulos de amido e desempenho de leitões [tese]. Botucatu (SP): Universidade Estadual Paulista; 2004.

Lumeij JT. Avian clinical biochemistry, In: Kaneko JJ, Harvey JW, Bruss ML, editor. Clinical biochemistry of domestic animals. San Diego: Academic Press; 1997. p.857-883.

Mahanna B. Proper management assures high quality silage grains. Feedstuffs 1994;10: 12-23.

Martinez KLA, Sartori JR, Costa C, Pezzato AC, Cruz VC, Pinheiro DF.Silagem de grãos úmidos de milho (C-806) na alimentação de frangos de corte em diferentes fases de criação. Anais do 21º Congresso Brasileiro de Zootecnia e 3º Congresso Internacional de Zootecnia; 2001; Goiânia, Goiás. Brasil. p. 40.

Martins CL, Cruz VC, Pinheiro DF, Sartori JR, Pezzato AC, Carrijo AS, Silva MDP. Silagem de grãos úmidos de milho na alimentação de frangos de corte: Peso de órgãos e morfometria intestinal. Revista Brasileira de Ciência Avícola 2000;2:49.

Marzzoco A, Torres BB. Bioquímica básica. In: Marzzoco A, Torres BB, editor. Metabolismo de lipídios. Rio de Janeiro: Guanabara Koogan; 1999. p. 194-215.

Medeiros CM, Baêta FC, Oliveira RFM, Tinôco IFF, Albino LFT, Cecon PR. Efeitos da temperatura, umidade relativa e velocidade do ar em frangos de corte. Engenharia na Agricultura 2005;4:13.

Meluzzi A, Primiceri G, Giordani R, Fabris G. Determination of blood constituents reference values in broilers. Poultry Science 1992;71:337-345.

Molnár-Perl, I. Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food. Journal of Chromatography A 2000;891(1):1-32.

Moran Júnior ET.Digestão e absorção de gorduras, In: Gonzales E, editor. Fisiologia, digestão e absorção de aves. Campinas: Facta; 1994. p. 71-82.

Nummer Filho I. Silagem de grão úmido de milho. Anais do 9º Seminário Nacional de Desenvolvimento da Suinocultura; 2001; Gramado, Rio Grande do Sul. Brasil. p. 28-42.

Oliveira Neto AR, Oliveira RFM, Donzele JL, Rostagno HS, Ferreira RA, Maximiano HC, Gasparino E. Efeito da temperatura ambiente sobre o desempenho e características de carcaça de frangos de corte alimentados com dieta controlada e dois níveis de energia metabolizável. Revista Brasileira de Zootecnia 2000;29(1):183-190.

Oliveira RFM, Donzele JL, Abreu MLT, Ferreira RA, Vaz RGMV, Cella PS. Efeitos da temperatura e da umidade relativa sobre o desempenho e o rendimento de cortes nobres de frangos de corte de 1 a 49 dias de idade. Revista brasileira de Zootecnia 2006a;35(3):797-803.

Oliveira GA, Oliveira RFM, Donzele JL, Cecon PR, Vaz RGMV, Orlando UAD. Efeito da temperatura ambiente sobre o desempenho e as características de carcaça de frangos de corte dos 22 aos 42 dias. Revista Brasileira de Zootecnia 2006b;35(4):1398-1405.

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Ferreira AS, Oliveira RF, Lopes DC. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Viçosa (MG): UFV; 2000. 141 p.

SAEG - Sistema de Análises Estatísticas e Genéticas. Versão 7.1. Manual de utilização do programa SAEG. Viçosa (MG): Universidade Federal de Viçosa; 1997. 150p.

Sahin K, Sahin N, Kucuk O.Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). Nutrition Research 2003;23(2):225-238.

Salgado DD, Nääs IA. Avaliação de risco à produção de frango de corte do estado de São Paulo em função da temperatura ambiente. Revista Engenharia Agrícola 2010;30(3):367-376.

Sartori JR, Costa C, Pezzato AC, Martins CL, Carrijo AS, Cruz VC, Pinheiro DF. Silagem de grãos úmidos de milho na alimentação de frangos de corte.Pesquisa Agropecuária Brasileira 2002;37(7):1009-1015.

Sartori JR, Gonzales E, Dal Pai V, Oliveira HN, Macari M. Efeito da temperatura ambiente e da restrição alimentar sobre o desempenho e a composição de fibras musculares esqueléticas de frangos de corte. Revista Brasileira de Zootecnia 2001;30(6):1779-1790.

Silva MAN, Silva IPO, Piedade SMS, Martins E, Coelho AAD, Savino VJM. Resistência ao estresse calórico em frangos de corte de pescoço pelado. Revista Brasileira de Ciência Avícola 2001;3:27-33.

Supelco Bulletin. Analyzing fatty acids by packed column gas chromatography [n.856B]. Philadelphia: Sigma-Aldrich Company; 1998. 12 p.

Wilson RK. A rapid accurate method for measuring volatile fatty acids and lactic acid in silage. RuaKura: Animal Research Institute; 1971. 12 p. (Research report).

Yahav S. The effect of constant and diurnal cyclic temperatures on performance and blood system of young turkeys. Journal of Thermal Biology 1999;24(1):71-78.

Yahav S, Shinder D, Tanny J, Cohen S. Sensible heat loss: the broiler's paradox. World's Poultry Science Journal 2005;61(3):419-434.

Zanusso JT. Níveis de energia metabolizável para frangos de corte de 1 a 21 dias de idade mantidos em ambiente de conforto e estresse térmico[dissertação]. Viçosa (MG): Universidade Federal de Viçosa; 1998.