Effect of Dietary Dehydrated Pasture and Citrus Pulp on the Performance and Meat Quality of Broiler Chickens

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ABSTRACT Some feedstuffs containing significant levels of fiber may be a good source of bioactive compounds that may contribute to improving broiler meat quality. However, high fiber level can have a negative impact on broiler performance. A study was undertaken to investigate the impact of incorporating citrus pulp (5 or 10%) or dehydrated pasture (5 or 10%) on the performance, carcass yield, and characteristics of broiler chickens. A diet containing neither citrus pulp nor dehydrated pasture was used as control. The results on growth performances showed that daily weight gain was reduced by 26% in birds of the 10% citrus pulp treatment ($P < 0.05$). Compared with the control treatment, increases in feed intake occurred in birds consuming diets with 5 or 10% citrus pulp, which resulted in significantly higher feed conversion rates with the 10% level. Under the same incorporation rate, dehydrated pasture had effects less evident on the performances of broiler chicken. In addition, diets containing citrus pulp, displaying higher percentages of soluble nonstarch polysaccharides, increased small intestine relative length, and reduced carcass yield. Inclusion of 10% dehydrated pasture in diets resulted in improved breast skin yellowness ($P < 0.05$). Finally, the results revealed that incorporation of the nonstarch polysaccharide-rich feedstuffs had a major impact on the fatty acid profile (affected 16 of 21 fatty acids) of broiler meat. Polyunsaturated fatty acids content in meat was higher in birds consuming the highest levels of both citrus pulp and dehydrated pasture, leading to increased ratios of polyunsaturated to saturated fatty acids. Together, the results suggest that incorporation of moderate levels of dehydrated pastures in poultry diets has a minor impact on broiler performance and can contribute significantly to improve breast skin yellowness and fatty acid composition of meat.

Key words: citrus pulp, dehydrated pasture, broiler chicken, fatty acid profile, polyunsaturated fatty acid/saturated fatty acid

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INTRODUCTION

Poultry diets are commonly corn/soy-based displaying a high energy concentration and low levels of fiber. However, a variety of feedstuffs with high fiber content have been commonly incorporated in poultry diets, particularly in extensive broiler production systems. Nevertheless, depending on the levels of solubility and concentration, fiber presence in diets affects poultry performance (Mathlouthi et al., 2002). Nonsoluble fiber, which encompasses insoluble nonstarch polysaccharides (**NSP**) and lignin, is usually regarded as a nutrient diluent. Therefore, insoluble fibers do not affect digestion and absorption of nutrients in the intestine (Carré, 1990; Edwards, 1995) and are not extensively degraded by bacteria in poultry, which makes their influence on the composition and quantity of the microflora relatively insignificant (Langhout, 1998). However, it has been suggested that fibers might encapsulate nutrients in the plant cell, becoming, therefore, a physical barrier to digestive enzymes (Pettersson and Aman, 1989). In contrast, soluble fiber contains soluble NSP, gums, and pectins, and the components of this feed fraction usually display antinutritive properties. Soluble NSP such as arabinoxylans and β-glucans are known to increase digesta viscosity, thereby affecting nutrient digestion and absorption (Smith and Annison, 1996) and reducing feed passage rate (Van der Klis et al., 1993). These effects may cause anaerobic microbial proliferation on the small intestine leading to the production of toxins and deconjugation of bile salts, which are essential for

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the digestion of fat (Misir and Marquardt, 1978; Langhout, 1998). Peptic substances are also soluble and have the ability to form gels and increase the digesta water holding capacity (Voragen et al., 1995, 2001). Moreover, decreased efficiency of nutrient utilization through microbial conversion of digestible carbohydrates to volatile fatty acids (Carré et al., 1995; Langhout et al., 1999) and deterioration of growth performance (Langhout and Schutte, 1996; Langhout et al., 1999) were observed when broiler chick diets included citrus pectins. Finally, it is widely accepted that dietary fiber can influence the development and the size of digestive organs in broilers chicks, which in turn has a significant influence in carcass yield. Diets with high levels of soluble NSP induced considerable enlargements of some portions of the gastrointestinal tract (Brenes et al., 1993; Petersen et al., 1999; Mourão, 2000) and pancreas and stimulated an increase in protein turnover rates (Dänicke et al., 2000). However, the effect of dietary insoluble NSP in the size of digestive organs is less clear (Taylor and Jones, 2001; Wu and Ravindran, 2004).

Low ratios of polyunsaturated fatty acids (**PUFA**) to saturated fatty acids (**SFA**) in Western diets have been considered as major risk factors for cardiovascular disease, which are among the most important causes of human mortality in developed countries (Hu et al., 2001; Ganji et al., 2003). In addition, PUFA contents of western diets are deficient in n-3 fatty acids leading to high n-6/n-3 fatty acid ratios (Simopoulos, 2002), which are responsible for the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. Moreover, it has been shown that consumption of eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) n-3 fatty acids may reduce the risk of coronary heart disease, and also are vital components in retina and membrane phospholipids of the brain (Rymer and Givens, 2005). Thus, it is widely acknowledged that there is an urgent need to return to a balanced fatty acid diet by improving the intake of polyunsaturated fats and n-3 fatty acids (Simopoulos, 2002). Poultry meat has been considered as one of the sources of PUFA, in particular n-3 PUFA, for human diets (Howe et al., 2006; Sioen et al., 2006). Some fiber-containing feedstuffs, such as dehydrated green pastures, are a good source of α-linolenic acid (**ALA**; 18:3n-3; Ponte et al., 2007). However, although pasture is a poor source of EPA and DHA, it is presently unknown if birds have the capacity to utilize pasture ALA as a precursor for the synthesis and deposition of these 2 fatty acids in broiler meat.

The increase in the degree of polyunsaturation of meat may promote the development of organoleptic problems due to a higher susceptibility to lipid oxidation (Bou et al., 2001). Antioxidants delay or prevent lipid oxidation by reducing free radical activities in meat. Therefore, antioxidant supplementation of feed is an efficient method for increasing meat oxidative stability (Maraschiello et al., 1999). The α-, β-, γ-, and δ-tocopherols, together with the corresponding tocotrienols (AOAC, 2000), are the natural diterpenes with vitamin E activity, which is the primary lipid-soluble antioxidant in most biological systems (Kerry et al., 2000), whereas tocotrienols are also known to help lower plasma cholesterol levels (Qureshi et al., 1997). Considering that the various vitamin E forms have different antioxidant potencies (Bourgeois, 1992) and biological activities, the quantification of all vitamin E molecules in foods is usually required. In addition, broiler carcass pigmentation is an important factor influencing consumer acceptance and perceived quality. Xanthophylls are lipid-soluble antioxidant pigments that after absorption are deposited in the skin and subcutaneous fat (Pérez-Vendrell et al., 2001). The intensity of pigmentation in broiler skin mainly relates to the total amount of xanthophylls ingested and their availability (Lipstein, 1989). Because carotenoids are transported in the intestine with the lipid phase, their solubilization and subsequent absorption is influenced by the nature and levels of dietary lipids (Furr and Clark, 1997; Garrett et al., 1999). Poultry cannot synthesize xanthophylls and must obtain these bioactive compounds directly from diets (Schiedt, 1998; Blanch, 1999). It is well known that a large range of forages, such as alfalfa, cocksfoot, perennial ryegrass, red clover, and grass meals, although containing considerable levels of fiber, are good sources of xanthophylls and could be used in poultry diets as pigmenting agents (Lipstein, 1989; Chauveau-Duriot et al., 2005).

The contribution of dehydrated pasture and citrus pulp to the nutrition of broiler chicks and the quality of the resulting poultry products remains largely unknown. Overall, this study aims to compare the effects of the diet incorporation of dehydrated pasture and citrus pulp, containing significant levels of insoluble and soluble fibers, respectively, on the growth performance and the yield, color, and chemical composition of carcass of broiler chicks.

MATERIALS AND METHODS

Diets, Animals, and Husbandry

Five dietary treatments were obtained by adding to a basal diet 50 g/kg or 100 g/kg of citrus pulp (treatments **5CP** and **10CP**) and 50 g/kg or 100 g/kg of dehydrated pasture (treatments **5DP** and **10DP**). The basal diet (control treatment) was a typical corn and soybean meal-based diet and was formulated to contain 3,000 kcal/kg of ME and to meet or exceed the NRC (1994) recommendations for all nutrients. Dehydrated pasture was obtained from a pasture based on Italian ryegrass (*Lolium multiflorum*) and balansa clover (*Trifolium michelianum*), harvested in the flowering state, and dehydrated. Generally, dehydrated leguminous-based pastures are moderately rich in protein and fiber but present low levels of energy and constitute a natural source of xanthophylls. Citrus pulp usually displays a high content in NDF (220 g/kg) and pectin (223 g/kg; Bampidis and Robinson, 2006). All the 5 diets were cold-pelleted (65 to 70°C). The ingredient composition of the experimental diets used on the study is presented in Table 1.

Table 1. Composition of dietary treatments on as-fed basis (%)

	Diet				
Ingredient	Control	5CP	10CP	5DP	10DP
Corn	55.8	53.0	50.2	53.0	50.2
Soybean meal	35.3	33.5	31.8	33.5	31.8
Soybean oil	4.9	4.7	4.4	4.7	4.4
Calcium bicarbonate	0.3	0.3	0.3	0.3	0.3
Calcium carbonate	1.5	1.4	1.3	1.4	1.3
Monocalcium phosphate	1.5	1.4	1.3	1.4	1.3
Salt	0.2	0.2	0.2	0.2	0.2
D,L-Methionine	0.2	0.2	0.2	0.2	0.2
Mineral and vitamin premix[1]	0.3	0.3	0.3	0.3	0.3
Citric pulp (CP)	0	5.0	10.0	0	0
Dehydrated pasture (DP)	0	0	0	5.0	10.0

[1]Mineral-vitamin premix provided the following per kilogram of feed: 11,000 IU of vitamin A, 2,150 IU of vitamin D_3, 25 mg of vitamin E, 1 mg of vitamin K, 5 mg of riboflavin, 20 mg of niacin, 8 mg of pantothenic acid, 1 mg of folic acid, 0.1 mg of biotin, 200 mg of choline, 0.012 mg of vitamin B_{12}, 50 mg of Mn, 40 mg of Zn, 5 mg of Cu, 0.1 mg of Se.

One hundred seventy 5-d-old male feather sexed broiler chicks (Ross 350) were fed a commercial rearing diet for 6 d. On d 6, birds were divided in 35 experimental replicates of 5 birds and allocated randomly into the 35 cages. Therefore, each treatment under analysis used 7 pens, making a total of 35 birds per treatment. The trial was carried out in accordance with the Portuguese law (Portaria n° 1005/92) on animal care in experimental research. The pens were located in a temperature-controlled room, which was daily adjusted to the recommended values, according to standard brooding practice. The chicks were given feed and water ad libitum throughout the experimental period (6 to 34 d). Body weight was recorded individually at 6 and 34 d of age. Body weight gain and feed intake were recorded on a pen basis and summarized for period from 6 to 34 d. Feed conversion ratio (**FCR**) was determined as the feed intake divided by the weight gain during the experimental period. Mortality was observed and recorded daily.

Carcass Characteristics and Digestive Tract Measurements

At the end of the experimental period, on d 34, 3 birds were selected from each replicate pen (closest to the mean pen weight) and fasted for 8 h. After fasting, body weights were recorded and the birds were killed by cervical dislocation followed by exsanguinations, semiscalded at 50°C for 2 min, and defeathered manually. After the removal of feathers, viscera, abdominal fat pad, shanks and neck, the weights of the eviscerated hot carcass and fat pad were measured. The gastrointestinal tract was carefully excised. The length of small intestine (from the pylorus to 1 cm above ileocecal junction) and cecum (left and right) were recorded. The carcasses were refrigerated for 24 h, and color of breast skin was individually measured using a Minolta CR-200 Chroma meter (Minolta Camera Co., Osaka, Japan). For each reading 3 measurements were performed, and the final value for each animal is the average of those readings. Skin color was expressed in the CIELAB dimensions of lightness (L*), redness (a*), and yellowness (b*). Meat pH was measured 24 h after cooling the carcasses. After carcass measurements, a skinless breast meat sample was collected from 1 carcass per pen, for determining total lipids, fatty acid composition, total cholesterol, and vitamin E compounds. The meat sample was ground using a food processor (3 × 5 s), vacuum packed, and stored at –80°C until required. In addition, 1 carcass per pen was cut into small pieces using an electric band saw and immediately ground in a mincer (Retsch SM 200, Haan, Germany) utilizing a sieve plate having holes of 8 mm in diameter. The mixture was then

Table 2. Nutritional composition of citrus pulp (CP), dehydrated pasture (DP), and experimental diets displaying different levels of the nonstarch polysaccharide (NSP)-rich feedstuffs[1]

	Ingredient		Diet				
Item	CP	DP	Control	5CP	10CP	5DP	10DP
Dry matter (% as-fed basis)	92.1	93.3	91.5	91.6	92.0	92.6	91.6
Organic matter (% DM)	92.4	86.7	93.6	92.6	93.4	92.9	92.2
NSPi (% DM)	20.5	27.5	10.5	10.8	11.6	11.7	13.6
NSPs (% DM)	11.9	4.8	1.4	2.3	4.2	1.7	1.9
Fat (% DM)	2.5	2.5	9.1	8.4	7.1	8.9	7.3
Protein (% DM)	8.1	13.6	22.2	22.9	21.5	20.6	20.4
Starch (% DM)	3.0	1.7	34.8	32.9	32.2	34.7	31.3
Free sugar (% DM)	4.1	2.6	4.6	1.9	3.8	3.1	4.5

[1]NSPi = insoluble nonstarch polysaccharides; NSPs = nonstarch polysaccharides.

ground through another sieve plate with 4-mm holes. Milled carcasses were homogenized in an industrial mixer (Stef, Rimini, Italy). One sample of approximately 100 g was placed in a sealed plastic bag and stored at –20°C for later chemical analysis.

Chemical Analysis

Proximal Analysis, Nonstarch Polysaccharides, and Starch. Ingredients, diets, and carcasses were analyzed for DM, ash, and crude protein made using the procedures of the AOAC (2000). Lipid analysis of ingredients and diets was performed by ether-extraction in a Tecator Soxtec HT 1043 (Höganäs, Sweden; ASTN, 1988). Insoluble nonstarch polysaccharides (**NSPi**) and soluble nonstarch polysaccharides (**NSPs**) were measured using the Englyst colorimetric method (Englyst and Cummings, 1988). Starch was determined by the enzymatic method described by Salomonsson et al. (1984).

Determination of Total Lipids. Meat samples were lyophilized (–60°C and 2.0 hPa) to constant weight using an Edwards Modulyo lyophilizer (Edwards High Vaccuum International, Crawley, West Sussex, UK), maintained exsiccated at room temperature, and analyzed within 2 wk. For total lipid determination, intramuscular fat was extracted as described by Alfaia et al. (2006) from the lyophilized samples (0.25 g). Total lipids were measured gravimetrically, in duplicate, by weighting the fatty residue obtained after solvent evaporation.

Determination of Fatty Acid Composition. Intramuscular fat of lyophilized samples (0.25 g) was initially dissolved in 1 mL of dry toluene. Fatty acids were converted to methyl esters (**FAME**) by base-catalyzed transesterification with sodium methoxide for 2 h at 30°C. The fatty acid composition was determined by gas chromatography of FAME with a Varian 3800 GC (Varian Inc., Walnut Creek, CA) equipped with a flame ionization detector and an OmegaWax 250 (Supelco, Bellefont, CA) capillary column (30 m × 0.25 mm i.d., 0.25-μm film thickness). The chromatographic conditions were as follows: injector temperature, 250°C; detector temperature, 280°C; helium was used as carrier gas, and the split ratio was 1:20. The gas chromatograph oven temperature was programmed to start at 150°C (maintained for 15 min) followed by a 3°C/min ramp to 220°C (maintained for 20 min). Peak identification was accomplished by comparing the retention times of peaks from samples with those of FAME standard mixtures. Quantification of FAME was based on the internal standard technique, using nonadecanoic acid (19:0) as internal standard, and on the conversion to relative peak areas to weight % using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed as gravimetric contents (mg/g of muscle) or as a percentage of the sum of identified fatty acids (% wt/wt).

Quantification of Total Cholesterol, Tocopherols, and Tocotrienol. The simultaneous determination of total cholesterol, tocopherols, and tocotrienols was performed as described by Prates et al. (2006). The method involves a direct saponification of meat (0.75 g), a single *n*-hexane extraction, and the analysis of the extracted compounds by normal-phase HPLC, using fluorescence (tocopherols and tocotrienols) and UV-Vis photodiode array (cholesterol) detections in tandem. The contents of total cholesterol, tocopherols, and tocotrienols were calculated, in duplicate for each sample, based on the external standard technique, from a standard curve of peak area vs. compound concentration.

Statistical Analysis

A completely randomized design was used in a monofactorial experiment. The statistical model included the effect of treatments (diets). A 1-way ANOVA with the GLM procedure was used to test for the effects of treatments on performances, carcass characteristics, digestive tract measurements, and meat composition. Differences among means were tested for significance ($P < 0.05$) using the Student's *t*-test. Statistical differences on mortality were analyzed using the chi-square test. All analyses were performed with SAS software (Version 8.2, SAS Institute Inc., Cary, NC).

RESULTS

The chemical composition of citrus pulp, dehydrated pasture, and experimental diets was determined and is presented in Table 2. Dehydrated pasture contained the highest percentages of total NSP (32% on a DM basis), of which the insoluble fraction is the most represented. As expected, citrus pulp presented lower levels of total NSP, although NSPs are more represented when compared with what was found in dehydrated pasture. Accordingly, the incorporation of dehydrated pasture and citrus pulp in the experimental diets mainly increased the contents of insoluble (NSPi) and soluble fiber (NSPs), respectively.

During the experiment the mortality was low (1.1%) and not related with any of the treatments. Body weight, weight gain, feed intake, and FCR of broiler chicks fed on diets containing various percentages of dehydrated pasture or citrus pulp are presented in Table 3. Inclusion of 5 or 10% of dehydrated pasture in the diets had no significant effects on final body weight and feed intake but reduced weight gain and at 10% level increased feed conversion ratio (**FCR**) when compared with birds fed the control diet. Incorporation of citrus pulp in the diets reduced birds' final body weight, and this effect was more pronounced with the diet containing the highest content of this pulp (**10CP** treatment). In addition, feed intake was higher in animals of the 10CP treatment, resulting in a significantly higher FCR. Overall, the results suggest that dehydrated pasture and citrus pulp in broiler diets affect animal performance in different ways and intensities. Whereas dehydrated pasture increases FCR, reducing growth without influence on feed intake, citrus pulp more intensely impairs FCR, improving feed ingestion and decreasing growth.

Table 3. Performances of broilers (6 to 34 d) feed on a cereal-based diet not incorporating (control) or containing citrus pulp (5% = 5CP, 10% = 10CP) or dehydrated pasture (5% = 5DP, 10% = 10DP)

	Treatment						
Item	Control	5CP	10CP	5DP	10DP	SEM	p(F)
Body weight (g)							
At 6 d	83.3	82.8	85.0	83.3	83.6	0.3	0.307
At 34 d	1,638^{a}	1,449^{b}	1,241^{c}	1,489^{ab}	1,497^{ab}	33	<0.001
Daily weight gain (g/d)	55.5^{a}	48.8^{b}	41.3^{c}	50.2^{b}	50.5^{b}	1.1	<0.001
Daily feed intake (g/d)	111.8^{b}	120.2^{b}	146.9^{a}	111.3^{b}	128.7^{ab}	3.6	0.003
Feed conversion ratio	2.058^{c}	2.505^{bc}	3.596^{a}	2.211^{bc}	2.554^{b}	0.127	<0.001

$^{a–c}$Means within the same row bearing different superscripts are significantly different ($P < 0.05$).

The effect of including NSP-rich feedstuffs in broiler diets on length of the small intestine and cecum was evaluated, and the collected data are presented in Table 4. As result of a small intestine with equivalent length in smaller birds, the small intestine relative length increased ($P < 0.05$) with the level of dietary citrus pulp, although it was not affected by the inclusion of dehydrated pasture. In addition, the length of the cecum was not affected by the treatments. The effects of including citrus pulp and dehydrated pasture in broiler diets on carcass traits were determined and are presented in Table 4. Birds from diets containing citrus pulp and dehydrated pasture presented lighter ($P < 0.05$) carcasses when compared with controls. In accordance with the performance data, 10CP birds presented the lighter carcasses of the treatments containing NSP sources and displayed significantly lower carcass yields. In addition, birds of the 10CP treatment presented lower levels of abdominal fat pad. The carcass composition was not influenced by the treatment. Results of the colorimetric evaluation of breast skin are presented as the CIELAB values of lightness, redness, and yellowness and suggest that although diets have no influence on lightness, there were considerable differences on redness and yellowness in the carcass skin of birds from different treatments. Although dehydrated pasture had no effect in broiler skin redness, it significantly increased skin carcass yellowness when diet contained 10% of dehydrated pasture ($P < 0.05$). Diets containing citrus pulp had no effect on yellowness, although it resulted in skin colors with reduced redness, especially in treatment 10CP. The 10CP and 10DP treatments reduced the intramuscular pH of poultry breast meat. Overall, the data suggest that whereas the 10DP treatment resulted in carcasses presenting more intense yellow tones, carcasses of the 10CP treatment displayed less intense red tones. Finally, as displayed in Table 4, inclusion of the NSP feedstuffs at the highest levels of incorporation (treatments 10CP and 10DP) resulted in reduced intramuscular pH of broiler breast meat.

Fatty acid composition of breast meat from broilers fed ad libitum on diets containing various percentages of citrus pulp or dehydrate pasture are presented in Table

Table 4. Effects of dietary citrus pulp (5% = 5CP, 10% = 10CP) and dehydrated pasture (5% = 5DP, 10% = 10DP) on carcass traits, skin color, meat pH, and relative length of small intestine and cecum of broiler chicken[1]

	Treatment						
Item	Control	5CP	10CP	5DP	10DP	SEM	p(F)
Carcass trait							
Weight	1,185^{a}	1,055^{a}	851^{c}	1,051^{b}	1,042^{b}	23	<0.001
Yield	712^{a}	701^{ab}	678^{c}	700^{ab}	695^{bc}	3	0.005
Abdominal fat	15.2^{a}	13.2^{ab}	10.9^{b}	14.6^{a}	15.4^{a}	0.6	0.048
Carcass composition							
Dry matter	359	332	342	347	347	29	0.546
Fat	117	92	95	98	108	3	0.070
Protein	230	229	236	240	229	1	0.160
Skin color[2]							
L	62.8	63.7	64.2	63.3	62.6	0.3	0.370
a	4.0^{a}	2.8^{bc}	2.6^{c}	3.6^{abc}	3.7^{ab}	0.2	0.048
b	10.2^{b}	9.9^{b}	10.6^{b}	11.5^{ab}	12.9^{a}	0.3	0.006
Meat pH	6.08^{ab}	6.10^{a}	5.93^{c}	6.06^{ab}	6.01^{bc}	0.02	0.001
Small intestine							
Total length	184	188	178	176	183	2	0.260
Relative length	112^{c}	127^{b}	143^{a}	119^{bc}	123^{bc}	2	<0.001
Cecum							
Total length	35.1	35.4	30.8	31.6	33.6	0.7	0.134
Relative length	21.4	23.8	24.6	21.3	22.4	0.6	0.248

$^{a–c}$Means within the same row bearing different superscripts are significantly different ($P < 0.05$).

[1]Measuring units: carcass weight (g), carcass yield (g/kg of BW), weight of abdominal fat pad (g/kg of BW), carcass composition (g/kg) and total (cm), and relative length (cm/kg of BW) of small intestine and cecum.

[2]L = lightness; a = redness; b = yellowness.

Table 5. Total lipids (mg/g of meat), total cholesterol (mg/g of meat), fatty acid composition (%Wt/Wt), partial sums of fatty acids (%Wt/Wt), and nutritional ratios of breast meat from broilers fed on cereal-based diet (control) or diets containing citrus pulp (5% = 5CP, 10% = 10CP) or dehydrated pasture (5% = 5DP, 10% = 10DP)[1]

	Treatment						
Item	Control	5CP	10CP	5PD	10PD	SEM	p(F)
Total lipids	5.11	5.18	5.29	5.14	4.61	0.26	0.391
Cholesterol	0.58^{ba}	0.60^{a}	0.54^{c}	0.58^{ba}	0.55^{bc}	0.01	0.008
Fatty acids							
14:0	0.24^{b}	0.59^{a}	0.25^{b}	0.31^{b}	0.24^{b}	0.09	0.044
15:0	0.07^{b}	0.13^{a}	0.08^{b}	0.09^{ab}	0.07^{b}	0.01	0.011
16:0	23.01^{ab}	24.55^{a}	21.39^{c}	24.14^{a}	22.07^{bc}	0.52	<0.001
16:1n-7	0.92	0.87	0.78	0.92	0.95	0.14	0.918
17:0	0.22^{c}	0.30^{a}	0.25^{b}	0.28^{a}	0.23^{bc}	0.01	<0.001
17:1	0.15^{a}	0.10^{b}	0.05^{c}	0.10^{b}	0.09^{b}	0.01	<0.001
18:0	15.73^{b}	16.87^{a}	14.76^{c}	15.30^{bc}	15.38^{bc}	0.25	<0.001
18:1n-9	20.81^{a}	19.26^{ab}	18.60^{b}	21.10^{a}	17.42^{b}	0.87	0.021
18:2n-6	21.11^{c}	20.69^{c}	24.54^{a}	20.97^{c}	23.04^{b}	0.49	<0.001
20:0	0.12^{b}	0.13^{a}	0.10^{c}	0.12^{ab}	0.11^{bc}	0.01	0.014
18:3n-6	0.05^{c}	0.07^{bc}	0.09^{a}	0.06^{bc}	0.08^{b}	0.01	<0.001
20:1n-9	0.29^{a}	0.25^{b}	0.26^{b}	0.31^{a}	0.31^{a}	0.01	0.003
18:3n-3	0.52^{b}	0.51^{b}	0.75^{a}	0.53^{b}	0.67^{a}	0.05	0.002
20:2n-6	1.23	1.25	1.22	1.33	1.45	0.08	0.200
20:3n-6	0.96^{b}	0.96^{b}	1.15^{a}	1.02^{b}	1.23^{a}	0.05	0.002
20:4n-6	9.07^{ab}	8.48^{b}	10.04^{a}	8.32^{b}	10.52^{a}	0.56	0.026
20:3n-3	0.13^{b}	0.14^{b}	0.16^{ab}	0.18^{a}	0.17^{a}	0.01	0.041
20:5n-3	0.22^{b}	0.20^{b}	0.26^{a}	0.23^{ab}	0.27^{a}	0.02	0.037
22:4n-6	2.32	2.05	2.25	1.19	2.47	0.16	0.145
22:5n-3	1.39	1.28	1.55	1.28	1.54	0.11	0.221
22:6n-3	1.03	1.11	1.24	1.16	1.36	0.13	0.463
Partial sums							
SFA	39.39^{b}	42.62^{a}	36.83^{c}	40.27^{b}	38.11^{bc}	0.53	<0.001
MUFA	22.57^{a}	20.65^{ab}	19.68^{b}	22.68^{a}	19.08^{b}	0.92	0.019
PUFA	38.05^{b}	36.79^{b}	43.3^{a}	37.08^{b}	42.82^{a}	1.07	<0.001
n-3	3.30^{b}	3.24^{b}	3.96^{a}	3.39^{b}	4.02^{a}	0.22	0.030
n-6	34.75^{b}	33.5^{b}	39.31^{a}	33.65^{b}	38.79^{a}	0.89	<0.001
Ratio							
PUFA/SFA	0.97^{b}	0.87^{b}	1.18^{a}	0.92^{b}	1.12^{a}	0.03	<0.001
n-6/n-3	10.91	11.26	10.06	10.52	9.93	0.57	0.435

[a–c]Means within the same row bearing different superscripts are significantly different ($P < 0.05$).

[1]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 = sum of 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3; n-6 = sum of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6; 22:2n-6, and 22:4n-6; PUFA/SFA ratio = sum 18:2n-6, 18:3n-6, 18:3n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3; 20:5n-3, 22:2n-6, 22:4n-6, 22:5n-3, and 22:6n-3/sum of 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0; n-6/n-3 ratio = sum of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6; 22:2n-6, and 22:4n-6/sum of 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3).

5. The predominant fatty acids in chicken meats of all treatments were palmitic (16:0) and stearic (18:0) acids as SFA, oleic acid (18:1) as monounsaturated fatty acid, and linoleic (18:2n-6) and arachidonic (20:4n-6) acids as PUFA. Nevertheless, palmitic, oleic, and linoleic acids were the most abundant fatty acids in the various meats under analysis. Consumption of the fibrous feedstuffs affected the concentrations of 16 of the 21 fatty acids analyzed, therefore affecting the partial fatty acid sums. These effects are clearly dependent on the level of inclusion of the fibrous feedstuffs. At a 5% incorporation rate of citrus pulp, an increase ($P < 0.05$) in the sum of SFA in relation to the control was observed, whereas at incorporation levels of 10% the levels of MUFA (mainly 18:1n-9) decreased ($P < 0.05$) and PUFA increased ($P < 0.05$) in relation to the control meat. Similarly, the inclusion of 5% of dehydrated pasture exerted only minimal effects on meat fatty acid composition, but when the inclusion was increased to 10%, a decrease ($P < 0.05$) in the percentage of MUFA (mainly 18:1n-9) and an increase ($P < 0.05$) in the levels of PUFA was observed. The increase in PUFA observed for the higher level of fibrous feedstuffs was due to the increase of both n-6 (mainly 18:2n-6 and 20:4n-6) and n-3 (mainly 18:3n-3) PUFA, so the nutritional relevant n-6/n-3 ratio was not changed.

Incorporation of citrus pulp or dehydrated pasture in diets for broiler chicks had no impact ($P > 0.05$) on breast meat total lipid content, as displayed in Table 5. In contrast, meat cholesterol varied among the treatments displaying the lowest and the highest values in birds of the 5CP and 10CP treatments, respectively. Nevertheless, dehydrated pasture had no influence in meat cholesterol contents, when these values were compared with those of meat from control animals. As expected, α-tocopherol, which coeluted in these meats with small amounts of α-tocotrienol, was the major vitamin E homologue detected in breast meats (Table 6). In addition, small contents of γ-tocopherol, which coeluted with a minor proportion of β-tocotrienol, γ-tocotrienol, and δ-tocopherol were also identified in the broiler breast meats. Although the levels of α-tocopherol remain unchanged in meats of the various treatments, inclusion of dehydrated pasture or citrus pulp

Table 6. Diterpenes (tocopherols and tocotrienols) contents (μg/g of meat) of breast meat from broilers fed on cereal-based diet (control) or diets containing citrus pulp (5% = 5CP, 10% = 10CP) or dehydrated pasture (5% = 5DP, 10% = 10DP; n = 35)

	Treatment						
Diterpenes	Control	5CP	10CP	5PD	10PD	SEM	p(F)
α-Tocopherol[1]	6.33	5.97	5.72	5.77	5.58	0.25	0.252
γ-Tocopherol[2]	2.088[a]	1.130[cd]	0.963[d]	1.678[b]	1.308[c]	0.088	<0.001
γ-Tocotrienol	0.371[b]	0.230[b]	0.819[a]	0.419[b]	0.465[b]	0.099	0.002
δ-Tocopherol	0.1172[a]	0.0639[b]	0.0491[b]	0.1103[a]	0.0932[a]	0.0103	<0.001

[a–d]Means within the same row with different superscripts are significantly different.

[1]Co-eluted with small amounts of α-tocotrienol.

[2]Co-eluted with small amounts of β-tocotrienol.

in the broiler diets led to a considerable decrease in the levels of meat γ-tocopherol. In addition, consumption of citrus pulp at low and moderate levels (treatments 5CP and 10CP) resulted in lower levels of δ-tocopherol. In contrast, meat from birds of treatment 10CP presented higher levels of γ-tocotrienol. Taken together, the results suggest that inclusion of citrus pulp and dehydrated pasture in broiler diets influenced meat composition in relation to the less represented vitamin E homologues.

DISCUSSION

In this study the effect of incorporating citrus pulp or dehydrated pasture in broiler diets on poultry performance, carcass yield, and meat quality was evaluated. Citrus pulp and dehydrated pasture presented similar levels of NSP; the sum of NSPi and NSPs amounts to 32.4 and 32.3%, respectively, in the 2 feedstuffs. The dehydrated pasture presented predominantly water-insoluble polysaccharides (85% of the total), whereas citrus pulp showed a much higher content in water soluble polysaccharides (37% of the total). However, the NSPs content of citric pulp was lower than the 223 g/kg of pectin referred to by Bampidis and Robinson (2006). It is possible that the colorimetric method used to measure NSPs (Englyst and Cummings, 1988) underestimates the fiber fraction when pectin content is high (Englyst et al., 1994). As expected, the incorporation of dehydrated pasture and citrus pulp in diets contributed to increase the levels of NSPi and NSPs, respectively.

Birds fed diets containing citrus pulp, which presented the highest levels of soluble NSPs, displayed impaired growth and high FCR. The negative effects were more evident when citrus pulp was incorporated at the highest level, although at 5% incorporation rate final body weights were significantly lower. In contrast, diet incorporation of dehydrated pasture did not have such negative effects on bird performance. However, final body weights of birds of the 5DP and 10DP treatments were marginally lower when compared with the controls. The negative effects of dietary fiber on broiler performances are intimately associated with a decrease in the digestion of nutrients and with a decrease of the diet AME (Tabook et al., 2006), which results from the birds' limited capacity to degrade NSP in the small intestine (Hesselman and Aman, 1986; Pettersson and Aman, 1989). In dehydrated pasture-containing diets, insoluble fiber acts primarily by diluting the nutrients concentration, increasing digesta passage rate and reducing nutrient digestibility (Hetland et al., 2004). In general, birds counteract these negative effects by increasing feed intake because it was marginally observed in birds of the 10DP treatment. Similarly, Hetland and Svihus (2001) and Tabook et al. (2006) observed high feed intake and no effects on performances when insoluble fiber was included at moderate levels in broiler diets. Therefore, birds are able to compensate for reduced nutrient concentration due to the presence of insoluble fiber by increasing feed consumption (Rogel et al., 1987), attenuating the potentially negative effects of a reduced digestibility on growth rate. In contrast, the addition of citrus pulp to the corn-based diet clearly contributed to depress weight gain and final body weight and to deteriorate FCR. Langhout and Schutte (1996) and Langhout et al. (1999) have reported similar negative effects of dietary pectins on broiler growth rates. However, here an increase in feed intake in birds receiving 10% of citrus pulp was observed, in contrast with what was previously reported in the above-mentioned studies. It was expected that diets with higher percentages of soluble fiber increased intestinal viscosity and, consequently, improved feed retention time in the gastrointestinal tract (Van der Klis et al., 1993). Because there is a negative relationship between feed retention time and feed consumption in young chickens (Almirall and Esteve-Garcia, 1994), diets with soluble fibers would contribute to reduced feed intake. Therefore, it is possible that the rise of relative length of small intestine in 10CP birds contributed to counteract this effect, allowing the increase of feed intake observed in this work. However, the viscous nature of the digesta may have resulted in a decreased nutrient digestibility (Hesselman and Aman, 1986), particularly that of fat (Langhout et al., 1999), resulting in a low AME (Annison, 1991) and in the poor productive value of the CP diets.

Diets containing citrus pulp, which are rich in soluble pectins, were shown to increase the small intestine relative length in 5CP birds and more significantly in birds of the 10CP treatment. Less evident effects were observed in animals disposing of dehydrated pasture-containing diets. Similar observations were made in previous studies using diets rich in soluble (Brenes et al., 1993; Petersen et al., 1999; Mourão, 2000) or insoluble fibers (Preston et al., 2000; Taylor and Jones, 2001; Wu and Ravindran, 2004). Therefore, the increased feed intake and the negligible digestion of dietary fibers contribute to an increase of the digesta bulk in the digestive tract. As a consequence, the organism reacted with an increased capacity of the digestive system (Brenes et al., 1993; Hetland and Svihus, 2001; Tabook et al., 2006) and through the induction of several physiological adaptations in an attempt to improve nutrient uptake [e.g., increased pancreatic enzyme secretion and absorptive area (Brenes et al., 1993; Tabook et al., 2006)]. In summary, because feed passage tends to be slower in diets containing viscous pectins or, more generally, in diets rich in soluble NSP, increased intestine relative length may constitute an adaptation mechanism to allow for an improvement in feed consumption and nutrient uptake. Finally, the data suggested that dietary citrus pulp negatively affected carcass weight and yield. Although birds consuming citrus pulp already presented an impaired performance, the more developed gastrointestinal tract clearly contributed to reduced carcass yield. Birds fed the diet with 10% citrus pulp presented a lower abdominal fat pad and a trend to a decreased carcass fat content. Therefore, lower growth rates presented by birds of the 10CP treatment were accompanied by a reduced fat deposition, confirming the observations of Summers et al. (1992) and Leeson and Zubair (1997). It is likely that birds were unable to obtain the required energy for normal growth and fat deposition. This energetic deficit manifested in broilers consuming citrus pulp was worsened by the increased energy expenditure as a result of the observed enlargement of the gastrointestinal tract. It has been previously reported that the increase of gastrointestinal tract size is intimately related with an increase of the energetic needs (Jorgensen et al., 1996).

Incorporation of citrus pulp and dehydrated pasture in broiler diets had a significant impact on the color profile of poultry breast skin. Overall, the data showed a reduction of breast skin redness in animals consuming the highest levels of citrus pulp, showing that the usually undesirable red tones in the skin were less developed. This observation could be due to low levels of red bioactive molecules in citrus pulp, reduced intestinal absorption of pigments, or both. It has been demonstrated that differential absorption of pigments may lead to differences in skin pigmentation (Castañeda et al., 2005). Pigment solubilization and subsequent absorption in the intestine occurs with the lipid phase (Furr and Clark, 1997; Garrett et al., 1999). Because soluble fibers negatively affect fat digestion and absorption (Silva and Smithard, 1997), one can expect a negative effect of citrus pulp fibers on the absorption of lipid soluble pigments. In contrast, diets with 10% dehydrated pasture significantly increased the yellowness of broiler breast skin ($P < 0.05$). The yellowness in breast skin is a good indicator of the xanthophylls content of the ingested feed (Pérez-Vendrell et al., 2001). Pastures are a well known good source of xanthophylls (Chauveau-Duriot et al., 2005; Nozière et al., 2006). Therefore, the more intense yellow tones of broiler carcasses from the dehydrated pasture treatments suggest a higher intake of yellow pigments, which may result from the intrinsic richness of these bioactive compounds in dehydrated pasture. The addition of 10% of citrus pectin to the diets reduced intramuscular pH of poultry breast meat. Nevertheless, the intramuscular pH values exhibited did not fall within the pH range typically associated with an unacceptable lean color. It has been suggested that the pale color of broiler skin in chicken can occur at a pH of 5.7 (Van Laack et al., 2000).

The inclusion of 10% of citrus pulp and dehydrated pasture consumption had a marked effect on the fatty acid profile of broiler meats. The modification involves a reduction in palmitic and oleic acids and an increase in n-6 and n-3 PUFA. These changes do not seem to be related with dietary contribution of both fibrous feedstuffs. Both citrus pulp and dehydrated pasture have low fatty acid content, although the concentration of linolenic acid (in % of total fatty acids) is consistently low in citrus pulp lipids (about 6%) and quite variable in dried forage lipids (30 to 60%). Moreover, most of structural lipids of pasture are probably not available for absorption, requiring fibrolytic fermentation not possible in the upper compartments of the broiler digestive tract. Consistently, the increase in α-linolenic acid proportion in relation to the control meat, although significant, is relatively small and higher for meats of birds of treatments CP10 (+0.21%) than for DP10 (+0.13%). Overall, the changes in fatty acid profile of broiler meats reported here most possibly result from the inhibition of de novo lipid synthesis (lower 16:0 and 18:1n-9) with an increase in proportion of exogenous fatty acids, mainly linoleic acid, abundant in the broiler diets. The inclusion of fibrous feedstuffs reduced the concentration of starch of the diets and might affect the energy status (supply of glucose), as suggested by the lower final weight and growth rate. It is well known that energetic status and the subsequent responses of metabolic hormones (insulin, glucagon, and 3,5,3′-triiodothyronine) are important factors that determine the level of hepatic lipogenesis in birds (Hillgartner et al., 1995; Richards et al., 2003). In chicken, rates of fatty acid synthesis in liver are low during starvation or feeding a low-starch diet (Wakil et al., 1983; Hillgartner et al., 1995), and insulin increases Δ^9-desaturase activity in hepatocytes (Lefevre et al., 1999). Also, Δ^9-desaturase expression in mammals is stimulated by the supply of glucose and insulin concentration (Ntambi and Miyazaki, 2004).

Dehydrated pastures are a good source of n-3 fatty acids (Ponte et al., 2007), but the levels of these fatty acids in meat from broiler chicken were unchanged among the treatments. Therefore, the n-6/n-3 ratio of broiler meats was not affected by the intake of citrus pulp or dehydrated pasture. In contrast, meat of birds from treatments 10CP and 10DP presented a higher value of PUFA leading to an higher PUFA/SFA ratio. Interestingly, the levels of ALA in broiler meat were improved as a consequence of dehydrated pasture or citrus pulp incorporation at the highest values. This observation suggests that the presence of higher values of the n-3 fatty acid precursor in the NSP-rich feedstuffs. In addition, levels of eicosapentaenoic acid were also increased in the meat of broilers consuming dehydrated pasture and citrus pulp at the highest levels of incorporation, suggesting that ALA is effectively desaturated and elongated in broilers of treatment 10CP and 10DP (Leece and Allman, 1996). However, the levels of the other nutritionally important n-3 fatty acids in meat, particularly of DPA and DHA, were not affected by dehydrated pasture of citrus pulp intake. In general the levels of the above-mentioned fatty acids in broiler meat are much lower when compared with the percentages of the long-chain n-3 fatty acids reported in meat originated in birds supplemented with 2 to 4% of fish oil (López-Ferrer et al., 2001). Therefore, these results suggest that supplementation of broiler diets with dehydrated pasture and citrus pulp is unable to substantially improve the n-3 fatty acids of broiler meat. Under these circumstances, direct supplementation with long-chain PUFA may be a more straightforward routine to improve meat nutritive value.

Data presented here revealed that all chicken meats are lean, based on the Food Advisory Committee (1990) criteria (<5% fat), and depict median contents of total cholesterol (0.54 to 0.59 mg/g). However, these variables were not affected by the incorporation of citrus pulp or dehydrated pasture in the diets. As expected, α-tocopherol, which coeluted in these meats with small amounts of α-tocotrienol, was the major vitamin E homologue detected in breast meats, although the values were not influenced by diet composition. The prevalence of α-tocopherol in meat is well known and is due to the more than 10-fold preference of the tocopherol-binding protein for α-tocopherol, relative to γ-homologes, which are the most common vitamin E molecules in plant foods (Decker et al., 2000). Surprisingly, levels of γ-tocopherol were significantly reduced in the meat of animals supplemented with citrus pulp and dehydrated pasture. A similar phenomenon was observed for the meat contents in δ-tocopherol, although this observation was restricted to the citrus pulp for this particular case.

In conclusion, incorporation of low (5%) or moderate (10%) percentages of citrus pulp in diets for broiler chickens increased the levels of dietary soluble NSP and led to impaired growth rates and FCR. Diets containing dehydrated pasture based on Italian ryegrass and balansa clover with high levels of insoluble NSP have effects on broiler performance less significant. High levels of soluble NSP in citrus pulp diets led to an increased relative length of the small intestine, contributing to reduced carcass yield. Even when incorporated at 5% level, dehydrated pasture was a good source of bioactive compounds that were effective in coloring the breast skin with more intense yellow tones. Finally, the results suggest that incorporation of citrus pulp or dehydrated pasture at the 10% levels changed meat fatty acid profiles, depressing MUFA and the palmitic acid and increasing the predominance of n-6 and n-3 PUFA. Our results suggest that dehydrated pasture may be an interesting supplement to include in broiler diets to improve meat quality with minor impacts in broiler performance at 10% of feed ingested.

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