ABSTRACT

There is an urgent need in industrialized societies to develop novel products that can lower human dietary cholesterol intake. Dehydrated alfalfa is a good source of hypocholesterolemic compounds such as saponins. Whether consumption of alfalfa by chickens would decrease the cholesterol content of broiler meat remains, however, to be established. We determined meat quality characteristics, lipid and cholesterol contents, and consumer preference of broiler meat derived from production systems based on restricted feeding of a commercial diet combined with provision of free-choice dehydrated alfalfa. Results demonstrated that it was possible to produce chicken breast meat with reduced cholesterol content. In addition, total lipids in chicken meat were significantly decreased when a higher level of restriction was applied. Members of a 50-person consumer panel preferred meat from animals not consuming or consuming moderate levels of alfalfa. Those members that preferred meat from animals consuming the higher percentage of alfalfa identified taste as the primary attribute influencing that decision. Together the results suggest that it is possible to develop novel broiler production systems that will produce leaner meat that is acceptable to consumers and has a reduced cholesterol content.

(Key words: alfalfa, broiler, cholesterol, consumer acceptance)

INTRODUCTION

Over the last century, the amount and proportion of animal fat in human diets has increased in many societies. These increases have been associated with the occurrence of cardiovascular diseases (Lichtenstein, 1999; Katan, 2000). In Western societies, coronary heart disease and arteriosclerosis are strongly related to the dietary intake of cholesterol and saturated fatty acids and are among the most important causes of human mortality (Sacks, 2002). In addition, a strong relationship has been demonstrated between cellular cholesterol concentration and Alzheimer’s disease (Michikawa, 2003). It is widely acknowledged that there is an urgent need to return to a balanced fatty acid diet by decreasing intake of cholesterol and saturated fatty acids, and there is still a paucity of information regarding production alternatives to achieve this important goal.

Alfalfa is widely used in animal feeding. It is moderately rich in protein but has low levels of energy. Dehydrated alfalfa meal generally is used at very low levels in poultry feeding, due primarily to its high fiber and low energy content. Alfalfa is also a natural source of xanthophylls, which when deposited in the skin and shanks give poultry carcasses a desirable yellow color (Dansky, 1971). In addition to its high fiber content, alfalfa contains high levels of bioactive antinutritive factors including saponins (Sen et al., 1998), which are steroid or triterpenoid glycosides. Saponins have been shown to have hypocholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant activities (Rao and Gurfinkel, 2000; Francis et al., 2002). The hypocholesterolemic

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Abbreviation Key: C50 = restriction to 50% of ad libitum consumption; C75 = restriction to 75% of ad libitum consumption; C100 = ad libitum consumption.
activity of saponins is well documented, with clearly defined molecular mechanisms. Some saponins form insoluble complexes with cholesterol in the digesta and inhibit the intestinal absorption of endogenous and exogenous cholesterol (Oakenfull and Sidhu, 1990). In addition, saponins can affect the enterohepatic circulation of bile acids by forming mixed micelles, which directly affect the reabsorption of bile acids from the terminal ileum (Oakenfull and Sidhu, 1990).

Garlic saponins can inhibit key enzymes in the cholesterol and lipid biosynthetic pathways (Konjufca et al., 1997). Whether saponins from other sources are similarly effective remains to be established. Alfalfa saponins prevent dietary hypercholesterolemia in rats (Story et al., 1984), and garlic saponins reduce cholesterol levels in plasma and meat in a variety of animal models, including the chicken (Chowdhury et al., 2002). Whether ingestion of alfalfa saponin can reduce cholesterol content in poultry meat remains to be established. Finally, very little is known about the sensory characteristics and consumer satisfaction of broiler meat produced using dietary interventions meant to reduce cholesterol content. The introduction of alfalfa in broiler diets could have major implications, not only in meat cholesterol levels but also in meat quality and consumer acceptance. It remains to be examined, however, whether alfalfa consumption affects the sensory characteristics of poultry products.

The objective of the research reported here was to establish if meat with reduced cholesterol and lipid contents could be produced from systems in which animals were fed restricted amounts of a commercial diet and allowed to consume alfalfa ad libitum. We also evaluated sensory characteristics of the meat obtained from the described production systems.

**MATERIALS AND METHODS**

**Animal Carcasses and Diets**

Male broilers (Ross 308) were fed with a high-energy commercial diet at 3 levels: ad libitum consumption (C100), restriction to 75% of ad libitum consumption (C75) and restriction to 50% of ad libitum consumption (C50). Animals on the 2 restricted diets had dehydrated alfalfa meal available for ad libitum consumption in separate feeders and grew in battery brooders exposed to constant light for the duration of the trial (5 pens per treatment with 8 broilers per pen). Broilers in the C100 group were raised in a conventional commercial production facility, under the same light and temperature program as the restricted-feed birds. In order that broilers on all 3 diets would have similar end carcass weights, the C100 group was started on feed 21 d later than the 2 restricted diet groups. Restricted and ad libitum broilers were grown at different locations as a biosecurity precaution. The ingredient composition and nutrient content of the commercial diet and the alfalfa used in these experiments are presented in Table 1. Water was available continuously throughout the experiment. Alfalfa intake was recorded weekly. At d 35 for the ad libitum birds and d 56 for birds on restricted feeding production systems, 25 broilers (5 birds per pen) of each treatment were slaughtered at a commercial processing plant. The carcasses were weighted and refrigerated for 24 h. Meat pH and water-holding capacities were measured as described by Sierra (1973). After these measurements, a breast meat sample was collected for cholesterol and lipid quantification, and carcasses were frozen at −20°C for posterior sensory evaluation.

**Analytical Procedures**

Total cholesterol was extracted from lyophilized meat (dry matter), after saponification with saturated methanolic KOH, according to the procedure of Naemi et al. (1995), except that 3 extractions with cyclohexane were used (recoveries were greater than 94%). Cholesterol was separated and quantified by normal phase HPLC, with an HPLC system equipped with an autosampler and diode array detector adjusted at 206 nm, a solvent (3% isopropanol in n-hexane) flow rate of 1 mL/min, and injection volumes of 30 µL. Total cholesterol content of each meat sample was calculated in duplicate, based on

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**TABLE 1. Ingredient composition and calculated analysis of the commercial high-energy broiler feed and dehydrated alfalfa meal**

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>High-energy feed</th>
<th>Alfalfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>58.18</td>
<td>—</td>
</tr>
<tr>
<td>Whole soybean</td>
<td>20.00</td>
<td>—</td>
</tr>
<tr>
<td>Soybean meal (47% CP)</td>
<td>17.60</td>
<td>—</td>
</tr>
<tr>
<td>Lard</td>
<td>3.40</td>
<td>—</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.89</td>
<td>—</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.80</td>
<td>—</td>
</tr>
<tr>
<td>Choline 75%</td>
<td>0.08</td>
<td>—</td>
</tr>
<tr>
<td>ti-Methionine</td>
<td>0.20</td>
<td>—</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Mineral and vitamin premix</td>
<td>0.60</td>
<td>100.00</td>
</tr>
</tbody>
</table>

1Mineral-vitamin premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D₃, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg; vitamin B₁₂, 0.12 mg; calcium pantothenate, 10 mg; vitamin K₃, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin B₂, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; butylated hydroxytoluene (BHT), 150 mg; monensin, 100 ppm.

2ADF = acid detergent fiber.

3NDF = neutral detergent fiber.
the external standard technique from a standard curve for peak area vs. concentration. Total lipids were extracted from meat (dry matter) and quantified by the Soxhlet method (Association of Official Analytical Chemists, 1980). Feed analyses for dry matter, ether extract, crude protein, and dietary fiber were performed according to methods of the Association of Official Analytical Chemists (1980).

### Sensory Evaluation

Carcasses were thawed at refrigerated temperature (4°C), slightly salted, and cooked for 40 min in a standard commercial oven at 200°C. Serving sizes were half a split breast piece served without the skin. The sensory panel consisted of 50 people, chosen from the staff of Estação Zootécnica Nacional, who were consumers of chicken meat and had participated in similar sensory evaluations. Panel members were not given any information about the meat or the experimental treatments and procedures. On the same day and at the same time, all 50 panelists were presented with a plate containing 3 numbered meat portions, 1 from a broiler with ad libitum access to standard commercial diet (C100) and 1 each from a C50 and a C75 broiler. Respondents were instructed to complete an evaluation form immediately following the meal and identify the meat sample that they most preferred and the basis for that decision.

### Statistical Analysis

Statistical analysis of meat quality, cholesterol, and lipid characteristics was conducted by one-factor analysis of variance using SAS software (SAS Institute, 2000) and, for data concerning consumer preferences, SPSS software (Statistical Package for the Social Sciences; Nie et al., 1975). Unless otherwise stated, differences were considered significant when \( P < 0.05 \).

### RESULTS AND DISCUSSION

Broilers on treatments C75 and C50 consumed cumulative averages of 986 and 2,540 g of alfalfa per bird, respectively, corresponding to 15 and 41% of their total feed intakes. Restriction of intake of a high-energy feed is, therefore, an effective means to induce alfalfa consumption by broiler chickens. The broilers did not exhibit any apparent health problems, although BW gains were reduced. Carcasses from C100 and C50 birds reached similar final weights; both were much lighter than C75 carcasses (Table 2).

### Meat Quality, Cholesterol, and Total Lipid

As shown in Table 2, neither age nor diet affected pH or water-holding capacities of breast meat measured after 24 h of refrigeration. Birds that consumed moderate (C75) or high levels of alfalfa (C50) produced breast meat with significantly lower cholesterol content than birds in the C100 group. Thus, bird age, presence of alfalfa in the diet, or a combination of these effects influenced total cholesterol content of breast meat. Several investigations have reported that cholesterol metabolism and cholesterol cellular concentration increase with age in poultry (Lorenz et al., 1938; Wagner and Clarkson, 1974; Qureshi et al., 1983). Because C50 and C75 birds were older than C100 birds but had lower cholesterol levels, alfalfa consumption, restriction of the high energy diet, or both were the factors most likely lowering cholesterol content in the meat. Interestingly, there were no differences in the levels of cholesterol in breast muscle from birds consuming moderate (C75) or high levels (C50) of alfalfa meal, suggesting that in these experiments, the main factor affecting meat cholesterol levels may have been the high-energy feed restriction. It is possible, however, that the effect is also mediated by alfalfa saponins and that this effect is not additive when going from C75 to C50. In both cases, the level of alfalfa saponins may have reached a required threshold to maintain cholesterol at the concentration observed. Further experiments will be implemented to differentiate between these two possibilities. Results suggest that it might be difficult, using alfalfa-based diets, to lower meat cholesterol levels to values below those reached in these experiments. Cholesterol

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**TABLE 2. Quality characteristics of breast meat from animals raised under a conventional production system (C100) or under restriction of the high-energy diet to 75% (C75) or 50% (C50) of ad libitum consumption**

<table>
<thead>
<tr>
<th>Production system</th>
<th>C100</th>
<th>C75(^1)</th>
<th>C50(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at slaughter (d)</td>
<td>35</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>High-energy feed intake (g)(^1)</td>
<td>3,035</td>
<td>5,632</td>
<td>3,653</td>
</tr>
<tr>
<td>Alfalfa intake (g)(^1)</td>
<td>0</td>
<td>986</td>
<td>2,540</td>
</tr>
<tr>
<td>Carcass weight (g)</td>
<td>1,739 ± 29.11(^\text{a})</td>
<td>2,431 ± 29.11(^\text{b})</td>
<td>1,727 ± 29.11(^\text{a})</td>
</tr>
<tr>
<td>Breast pH</td>
<td>5.50 ± 0.020</td>
<td>5.48 ± 0.020</td>
<td>5.47 ± 0.020</td>
</tr>
<tr>
<td>Water-holding capacity</td>
<td>35.97 ± 1.27</td>
<td>32.91 ± 1.27</td>
<td>34.40 ± 1.27</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>25.46 ± 0.013</td>
<td>25.75 ± 0.004</td>
<td>25.51 ± 0.002</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>2.29 ± 0.572(^\text{a})</td>
<td>2.84 ± 0.990(^\text{b})</td>
<td>1.74 ± 0.590(^\text{b})</td>
</tr>
<tr>
<td>Cholesterol (mg/100 g)</td>
<td>47.11 ± 3.14(^b)</td>
<td>43.18 ± 1.73(^a)</td>
<td>43.74 ± 1.49(^a)</td>
</tr>
</tbody>
</table>

\(^\text{a,b}\)Means within each row followed by different superscripts are significantly different \((P < 0.05)\).

\(^\text{1}\)C75 and C50 broilers voluntarily consumed moderate and high amounts of dehydrated alfalfa meal, respectively. Results are a mean ± standard deviation of 25 birds per group.

\(^\text{2}\)Average cumulative high-energy feed and alfalfa intakes per bird.
is an important molecule that has roles in membrane structure as well as being a precursor for the synthesis of molecules such as steroid hormones, vitamin D, and bile acids. Cholesterol can be obtained directly from the diet, or it can be synthesized in cells from 2-carbon acetate groups of acetyl-coenzyme A. Because the synthetic pathway is under feedback control from dietary cholesterol, the percentage of cholesterol arising from de novo biosynthesis or from the diet depends upon the amount of cholesterol that is ingested. Even when cholesterol intake is very low, de novo biosynthesis will enable the production of the cholesterol required to supply the large variety of biological processes in which this molecule is involved. Therefore, under these conditions only a direct alteration of the cholesterol biosynthetic pathway would enable a more radical alteration of the final cholesterol levels of broiler meat. Results of the current experiment suggest that factors that contribute to lowering cholesterol concentration are not affecting the in vivo biosynthesis of cholesterol; levels of meat cholesterol were identical in birds from treatments C75 and C50. These data are in contrast with the current evidence that garlic supplements have a dramatic effect on the cholesterol biosynthetic pathway (Konjufca et al., 1997). Those authors reported cholesterol values of breast meat to be as low as 38 mg/100 g using a 3% supplementation with garlic. Garlic might, therefore, be a better supplement than alfalfa to produce poultry meat with lower cholesterol levels. However, garlic might alter the sensory characteristics of poultry meat, which could have a major impact on consumer acceptance (Konjufca et al., 1997).

Finally, although cholesterol content of meat derived from C50 and C75 animals was similar, the total lipid content of breast meat originating from birds subjected to the higher feed restriction (C50) was significantly lower than that of any other treatment (Table 2). This decrease might be related to nutritional stress imposed by feed restriction rather than from alfalfa intake per se. Results indicate that this production system (C50) could be implemented to produce leaner meat with low cholesterol content. Producing broiler chicks under the systems used for C75 and C50 might be unprofitable, unless meat produced from these systems could be sold at a premium. Due to the feed restrictions imposed, quantities of high-energy feed required for systems using alfalfa meal would not be significantly higher than in conventional systems, when aiming for a broiler final BW of 2,000 g (data not shown).

**Sensory Evaluation**

Approximately equal numbers of sensory evaluation panel members preferred breast meat from C100 and C75 birds (20 and 19, respectively) with fewer (11) preferring meat from the C50 group. A cross-table relating preference for meat of the 3 treatments to attributes cited as responsible for the preference decision was produced (results not shown). This cross-table provided a contingency coefficient equal to 0.614, meaning that there was a non-random association between a preferred meat and the reasons for choosing it. Taste was the most frequently cited attribute (19/47) affecting consumer selection. This observation is interesting because tenderness is usually assumed to be the most important organoleptic characteristic of meat (Seabra et al., 2001).

By using these results and following Lebart et al. (1984) and Hair et al. (1992), we used correspondence analysis to draw a perceptual map of the attributes most frequently associated with preference of meat from each of the 3 sources (Figure 1). Hoffman and Franke (1986) argue that correspondence analysis, although not appropriate for hypothesis testing, is useful to map multivariate categorical relationships without strict data requirements. It enables the perceptual mapping of objects relative to a set of attributes. In the perceptual map, the degree of association between categories is inversely proportional to the distance between them (Hair et al. 1992). The representation (in Figure 1) suggests that panel members preferring meat from the commercial production system (C100) did so mainly because of its tenderness and less often because of tenderness and juiciness. Surprisingly, taste and taste plus tenderness are characteristics cited more often by panelists preferring meat from the C50 and C75 birds. Although C50 meat was preferred by only a few consumers, their selection was made predominantly on the basis of taste. It is well established that meat flavor intensifies with poultry age (Ramaswamy and Richards, 1982), suggesting that the reported effect on sensory characteristics of meat from treatments C50 and C75 was mainly due to broiler age. Nevertheless, the results suggested that alfalfa consumption did not create off-flavors in poultry meat, such as have been reported for meats from broilers fed on diets containing fish products (Lopez-Ferrer et al., 1999). It remains to be established whether differences in the number of consumers selecting C50 and C75 meats results from the lower lipid content of C50 meat or from the higher alfalfa intakes of the C50 chickens.
In conclusion, we have shown that novel broiler production systems can be implemented to produce poultry meat with lower cholesterol and lipid contents than from traditional production systems and that meat from broilers fed moderate levels of alfalfa is acceptable to consumers. Consumers might, however, be less satisfied with meat derived from broilers consuming high levels of alfalfa. It remains to be determined whether this results from the lower fat content of C50 meat or from the presence of potential off-flavors caused by the higher percentage of alfalfa in the diet of C50 chickens.

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REFERENCES


