

1 Effect of groundnut cake substitution by glandless cottonseed kernels on broilers production:
2 animal performance, nutrient digestibility, carcass characteristics and fatty acid composition
3 of muscle and fat.

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5 Mamadou Tandiang Diaw¹, Abdoulaye Dieng¹, Guy Mergeai², Ibrahima Youssouf³, Jean-
6 Luc. Hornick⁴

7 ¹ University of Thiès, UFR SADR, Department of Animal Production, Senegal

8 ² Gembloux Agricultural University, Department of Tropical Crop Husbandry and
9 Horticulture, Belgium

10 ³ Abéché University of Sciences and Technics, Tchad

11 ⁴ University of Liege, Faculty of Veterinary Medicine, Department of Animal Production,
12 Belgium.

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14 Address for communication: J.-L. Hornick, University of Liege, Faculty of Veterinary
15 Medicine, Department of Animal Production, Boulevard de Colonster B43, 4000 Liège,
16 Belgium, Fax: +3243664122, Tel: +3243664131, Email: jlhornick@ulg.ac.be

1 Abstract

2 A study has been conducted with broilers to assess, during the rainy season, the effects of
3 groundnut cake substitution by glandless cottonseed kernel (CSK), at levels of 0, 25, 50 and
4 75%. The substitution improved linearly feed intake and animal growth, as well as carcass
5 component weights and allometric parameters. The CSK increased the C18:2 n-6 to C18:1 n-9
6 ratio, as well in diet as in meat and subcutaneous fat. In order to explain the observed
7 performances, the possibility is considered that broilers used preferentially C18:2 n-6 fatty
8 acids for their metabolism. Complete glandless cottonseed kernels are probably highly
9 valuable for broilers production in warm and wet conditions.

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11 Key words: Groundnut cake; cottonseed kernels; glandless; broilers

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13 Short title: Glandless cottonseed kernels in broilers

1 Introduction

2
3 Cotton is the second best protein-producing plant in the world after soybean and the seeds are
4 potentially a valuable feed for poultry. The presence of the terpenoid aldehyde gossypol,
5 particularly noxious for monogastrics (Lusas and Jinidin, 1987; Alford et al., 1996; Morgan et
6 al., 1988; Willard et al., 1995), prevents however the extensive use of cottonseed by-products
7 in this species (Azman and Yilmaz, 2005), inasmuch as they are often high in cellulose
8 (Gamboa et al., 2001; Ojewola et al., 2006).

9 Some decades ago, a cotton mutant totally devoid of gossypol has been discovered but despite
10 the high nutritive value of the seeds, efforts ended up in commercial failure because of the
11 plant's susceptibility to insects (Altman et al., 1990).

12 Breeding efforts are now being made in different parts of the world to develop cotton varieties
13 presenting gossypol glands in their aerial organs but not in the seeds (Vroh bi et al, 1999;
14 Sunilkumar et al., 2006). The progresses made in this field could lead to the production of
15 cotton seeds available for monogastric.

16 Literature reporting the use of cotton cake in poultry exists (Ojewola et al., 2006;
17 Nagalakshmi et al., 2007). That concerning the effects of glandless varieties is, as for it,
18 scarce (Yo, 1991). Like cottonseed meal, cotton seed kernels (CSK) are high in protein, but
19 they are also naturally high in lipids, low in fibre, and they do not require high technicality to
20 be obtained. Moreover, lipids are intracellular and are thus presumably better protected
21 against oxidation than oils offered as energy source in poultry.

22 The aim of this experiment was thus to study, in broilers, the effects of groundnut cake
23 substitution by increasing levels of CSK deprived of gossypol glands on animal performance,
24 nutrient digestibility, carcass characteristics and fatty acid composition of broilers' muscle and
25 subcutaneous fat.

Materials and Methods

The trial was approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Liège (Belgium). The experiment was planned at the end of the rainy season 2006, at the Experimental Station of the Formation and Research Unity of Agronomic Science and Rural Development of the University of Thiès (Senegal).

During the experiment, temperature and moisture were daily recorded at 07.00 a.m., 01.00 p.m. and 06.00 p.m. The mean temperature was 31.0°C, with minima and maxima of $26.8 \pm 2.2^\circ\text{C}$ and $34.1 \pm 2.0^\circ\text{C}$ measured respectively at 07.00 a.m. and 01.00 p.m., and mean moisture was 60.2%, comprised between $48.0 \pm 9.7\%$ and $71.5 \pm 10.7\%$.

Four hundred 1-d-old unsexed and unidentified Cobb 500 broilers were used in this experiment. They were randomly assigned to four groups: a control group (CG) that received a diet containing groundnut cake as main protein source, and 3 other groups that received diets in which groundnut cake was substituted with increasing levels of shelled gossypol-free cotton seed kernels produced from *Gossypium hirsutum* cultivar GL7 (25, 50 and 75% substitution for G25, G50 and G75 groups, respectively). Each group was divided in two homogenous blocks.

A starting diet was used until 21 days old and a growing diet was offered afterwards until slaughter at d45. The different diets were formulated to present theoretical iso-proteic and iso-energetic characteristics. Iso-EE diets levels were ensured by the use of groundnut oil addition, with respect to the levels of substitution. Consequently, levels of nutrients were that recommended by the National Research Council (NRC, 1994), but ME that was expected to be slightly higher than classical recommendations. Feed and water were provided on a marginal *ad libitum* basis for the duration of the experiment.

1 Individual body weights (BW) were obtained on d1 and once a week thereafter. Birds were
2 observed twice daily to assess healthiness and death occurrence. Feed intake (FI) was
3 recorded weekly.

4 At the end of the experiment, 5 animals par group were randomly chosen and killed by
5 cervical rupture. They were eviscerated for carcass characteristics determination. Individual
6 weights of carcass, breast, legs and wings were measured. Samples of breast muscle and
7 subcutaneous adipose tissue were obtained on each carcass and frozen for further chemical
8 analyze.

9 Nutrient digestibility was evaluated with five additional 6-w-old Cob 500 broilers, mean BW
10 of 1300 g, for each of the 4 diets and two periods. They were penned in individual
11 metabolism cages and, after an adaptation period, feed intakes, refusals and faeces were
12 obtained once a day over a period of 7 d. Nutrient digestibility was calculated as the ratio
13 (nutrient intake – faecal nutrient) / nutrient intake.

14 The prophylactic program was the one used in poultry production in Senegal and during the
15 experimental period no sanitary trouble was identified.

16 The amino acid composition of the cotton seeds was obtained by liquid chromatography and
17 compared to that of the groundnut as reported by the literature (Henry et al., 2001), allowing
18 thus subsequent formulation. Aflatoxins levels (B1 , B2, G1 and G2) were determined in
19 groundnut cake and in the control diets by liquid chromatography according to the 92/95 and
20 94/14 directives of the European Commission. Total and free gossypols in diets were
21 determined according to the 72/199/CEE directive of the European Commission.

22 Dry matter (DM), ash, crude fiber (CF) and ether extract (EE) were analysed according to
23 AOAC (1990) procedures. Crude protein was determined by the Kjeldahl method, as nitrogen
24 (N) x 6.25. Calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and copper (Cu) were
25 determined by atomic absorption and potassium (K) and sodium (Na) by flame emission.

1 Total phosphorus (P) was determined by spectrophotometry. Sample solutions were prepared
2 using wet digestion procedure.

3 Metabolizable Energy (ME) was calculated by an indirect method, using the INRA equation
4 (1984) where True ME (Mj/kg DM) = (3951 + 54.4 EE – 88.7 CF – 40.8 ash)*0.004184,
5 where nutrient contents are expressed in % DM.

6 The determination of the fatty acid profile of diets, meat and fat was performed using gas
7 chromatography (GC) after extraction and trans-esterification of fatty acids according to the
8 method of Sukhija and Palmquist (1988). A combined one-step extraction and esterification
9 method was carried out using a mixture of solvents containing methanol, benzene and acetyl
10 chloride, to produce the different fatty acid methyl esters. The internal standard was
11 nonadecylic acid (C19:0). A 1 µl aliquot was injected into a Chrompack CP 9001
12 chromatograph (Middelburg, The Netherlands) fitted with a CP-9010 automatic liquid
13 sampler, a split-splitless injector and a 901A flame ionization detector (Chrompack,
14 Middelburg, The Netherlands). The GC system was fitted with an Omegawax 320 fused silica
15 capillary column (30 m x 32 mm i.d.) with a stationary polyethylene glycol phase (Supelco,
16 Bellefonte, United States of America) coated with a 0.25 µm film thickness. Hydrogen was
17 used as carrier gas at a pressure on the top of the column of 50 kPa. The column temperature
18 was programmed from 120 to 240 °C at a rate of 5 °C/min. The temperatures of the injection
19 port and detector were 250 °C and 260 °C respectively. The injection was performed in the
20 split mode with a split ratio of 1:25. The software Alltech Allchrome Plus Chromatography
21 Data System Version 1.4.2.1, Alltech Associates Inc., Lokeren, Belgium) was used for data
22 processing. Fatty acids were identified by comparison of their retention times with that of the
23 corresponding standard mix (Supelco 37 Component FAME Mix, Sigma-Aldrich, Bornem,
24 Belgium).

1 The data were analysed according to General Linear Models (SAS Institute, 1999). The level
2 of CSK incorporation was the main effect considered. When possible, the block and the
3 interaction level of incorporation x block effects were estimated and considered as random.
4 Owing to the variability in muscle ether extract, muscle fatty acids concentrations were
5 analysed using muscle ether extract as covariable. The fatty acids proportions in subcutaneous
6 and muscle fats (% fat) were analysed using the level of CSK incorporation, the fat
7 localisation (muscle or subcutaneous fat) and their interaction as factors of variation.
8 Means were compared according to Student's t-test.

Results

The only significant B1 aflatoxin level in groundnut cake was found to be low, at 0.13 mg/kg feed, allowing levels as low as 0.037 mg/kg in control diet. The amino acid profile of the glandless cottonseed kernels used in this experiment was similar to that classically reported for cotton cake (Table 1). Methionine and Lysine levels were close to that reported by Amipig (2000) for the groundnut cake (respectively 5.2 and 16.8 vs 4.7 and 12.4 g/kg feed).

As expected, the high fat levels in diets, close to 100g/kg, increased ME levels above values currently reported in poultry production (Table 2). Total gossypol remained largely lower than the critical levels reported for poultry in the literature (Lordelo et al. 2005). Free gossypol, as for it, was not detected.

In decreasing importance, C18:1 n-9, C18:2 n-6 and C16:0 accounted for about 90% of the total fatty acids of the diets (Table 3). The main characteristic of the fat diets was an inversion of the ratio C18:1/C18:2 between the extreme regimens (about 1.6 for CG to about 0.7 for G75). The saturated fatty acids (SFA) levels remained close to 20%. Consequently, a shift from a dominant monounsaturated (MUFA) to a dominant polyunsaturated (PUFA) profile was observed when turning from control to G75 diet.

The BW at the end of the starting and of the growing period was significantly ($P < 0.001$) affected by the level of substitution ($P < 0.001$; Table 4). Control group reached weight close to 1100 kg at the end of the experiment vs 1900g in G75. The linear correlation between final live weight and level of substitution allowed to estimate final BW for total groundnut substitution at 2211 g.

ADG and FI were sharply affected by the level of substitution. ADG increased quasi-linearly with the level of glandless seeds, as well as during the starting than during the finishing period. FI almost doubled when turning from CG to G75 groups, whatever the considered

1 period. Intra-period FCR were similar between groups, although they decreased with the level
2 of CSK substitution during the growing period (2.39 in G75 vs 2.72 in CG).

3 Nutrient digestibility ranged between 65 to 74%, according to the nutrient, and was not
4 affected by the treatments (Table 5).

5 Owing to the effect of CSK on final weight, the weight of carcass and of carcass components
6 increased significantly with CSK incorporation (Table 6). However, the weights of liver, heart
7 and gizzard did not change. As a consequence, the dressing proportion increased, as well as
8 the ratio of the breast to carcass ($P < 0.05$). Other ratio did not differ between treatments.

9 Ether extract in meat DM ranged from 63 to 77 g/kg in CG and G75, respectively, and was
10 similar between groups (Table 7). The SFA almost doubled from CG to G75 ($P < 0.002$)
11 while MUFA showed opposite evolution. The PUFA increased with CSK incorporation but to
12 a lesser extent (13.7 vs 18.4 g/kg DM in CG and G75 respectively, $P < 0.015$). As a
13 consequence, the ratios having SFA as denominator decreased significantly with CSK
14 incorporation, excepted for PUFA/SFA (NS), while PUFA/MUFA increased (more than
15 doubled), as did the ratio n-6 to n-3 fatty acids.

16 Considered individually, SFA increased with CSK incorporation but C16:0, followed with
17 C18:0, represented far the highest fraction of this family. Individual MUFA behaved as total
18 MUFA but C18:1 represented far the largest fraction of this family. Among PUFA, C18:2 n-6
19 accounted for about 75% of total PUFA. Only C18:2 n-6, C18:3 n-6 and 18:3 n-3 increased
20 significantly with CSK incorporation. However, although total n-6 fatty acids increased with
21 treatment, total n-3 fatty acids remained as low as about 9.5 g/kg muscle DM (NS).

22 The fatty acids proportions in subcutaneous and muscle fat are given in Table 8. The main
23 fatty acids found in animal fats were, by decreasing importance, C18:1, C16:0, C18:2n6 and
24 C18:0. Together, they represented 90% of the total fatty acids identified. The CSK
25 incorporation increased the proportions of SFA in subcutaneous and, especially, in meat fat (P

1 < 0.05 for the interaction effect). The mean values changed from about 30% of fat in CG to
2 42% in G75. By contrast, MUFA proportion decreased in opposite sense, ranging from about
3 45 to 50% in CG to 42% in G75, the location effect being not significant. The PUFA
4 proportions were influenced only by the level of substitution: the levels increased from about
5 21% in CG to about 25% in G75. The sum of n-6 fatty acids behaved closely to PUFA and the
6 sum of n-3 fatty acids were not influenced by treatment. Its levels were however trebled when
7 turning from subcutaneous fat to meat fat. As a consequence, the ratios containing SFA as
8 denominator decreased with CSK incorporation, especially MUFA/SFA, and were higher in
9 meat fat. By contrast, PUFA/MUFA increased with CSK incorporation while its values
10 remained higher in meat. The ratio n-6 to n-3 fatty acids increased with CSK incorporation
11 and to a largely higher extent in subcutaneous fat.

12 Taken individually, the fatty acids were essentially represented, in both fat and in decreasing
13 order, by C18:1, C16:0 or C18:2 n-6, C18:0 and C16:1. The C14:0, C16:0 and C18:0 fatty
14 acids behaved as SFA - increasing with CSK incorporation - the extent of changes being the
15 most marked for C18:0 in subcutaneous fat and similar between fatty acids in meat fat.
16 Among MUFA, C18:1 represented the very larger proportion and thus showed, with CSK
17 incorporation, a decrease similar to total MUFA. This decrease was higher in meat fat.
18 Among PUFA, C18:2 n-6 represented the very larger proportion. It showed noticeable
19 changes, increasing with CSK incorporation, but the increase was not significant in
20 subcutaneous fat and marked in meat fat. The C18:3 n-3 proportion, although weak in both
21 fats, decreased significantly with CSK incorporation in subcutaneous fat and increased in
22 meat fat.

Discussion

There are few recommendations concerning fat levels in poultry feeding but chicken are able to tolerate very high proportions of neutral fat in the diet (Brambila and Hill, 1966). In the present experiment, the levels of 10 to 12 % EE in diet were thus compatible with fattening. Fat sources are either useful for poultry feeding under warm climates because they allow compensating the drop in voluntary feed intake observed when temperature is high (Nahashon et al., 2006).

The lack of significant gossypol levels in the diets, especially free gossypol, indicates that the CSK used in this experiment was safety for poultry and was a good source of energy, owing to their high levels of fat, at about 37 % of the DM.

The final BW of the control groups was weak when considering the standard values reported for Cobb (2004). Aflatoxins incidence could be neglected since their levels were largely lower than the levels of 1 mg/kg causing a 5% reduction in growth rate in poultry, as reported by Dersjant-Li et al. (2003). The low growth of the CG was probably due to the fact that the experiment was carried out at the end of the rainy season under hot and wet climate, i.e., suboptimal conditions for broiler production. Indeed, over 30°C, FI decreases drastically in order to limit endogenous heat production, reducing growth performances and thus final BW (Dale and Fuller, 1979). Cooper and Washburn (1998) yet shown that broilers exposed to 32°C expressed almost half the growth performed at 21°C. Considering the iso-nutrient conditions scheduled in this experiment, the effects of CSK substitution on animal growth and on carcass characteristics, with emphasis the higher development of breast, are thus surprising. The higher performances were clearly associated with higher FI, but not with better FCR. This firstly indicates the high palatability of glandless CSK for poultry. The amino acid profile was assumed similar between groundnut and CSK proteins. The most

1 significant difference between diets was the ratio of CSK to both groundnut cake and
2 groundnut oil. Consequently, the effects of the treatments could be ascribed to differences in
3 fatty acid profile. Grains are generally rich in linoleic acid (Watkins, 1991), and poultry are
4 adapted to grain intake. The shift in fatty acid profile from C18:1n-9 to C18:2 n-6 might thus
5 explain the increased diet palatability.

6 Possibly, it also modified fatty acid metabolism. Some fatty acids have specific effect on
7 metabolic pathways. These aspects are poorly known in poultry and are largely based on
8 mammalian models. Briefly, the n-6 family promote the pro-inflammatory arachidonate way
9 while n-3 family promote the pro anti-inflammatory eicosapentanoate one. These ways have
10 specific effects on animal growth (Watkins 1991).

11 Theoretically, in this experiment the C18:1 n-9/C18:2 n-6 shift promoted the arachidonate
12 way and thus the fatty acids from n-6 family. However, excepted for C18:3 n-6 concentration
13 in muscle DM, no detectable effect of the treatment could be observed on fatty acids levels
14 from n-6 family. The differences in animal weight and carcass characteristics could be thus
15 hardly ascribed to differences in n-6 vs n-3 metabolic pathways.

16 According to a feed conversion ratio close to 2, to the approximatively 110g fat /kg diet
17 offered to birds in this experiment, and to a fat digestibility of about 70% (see tables), the
18 animals ate about 154 g digestible fat par kg live weight. Considering 15 g fat/kg muscle (i.e.,
19 about 60 g fat/kg muscle DM, table 7), body muscle proportion of about 750 g/kg, and 100 g
20 adipose tissue/kg body, or about 80-120g total fat/kg body weight (Mitchell et al., 1997),
21 animals fixed less fat than amounts digested, in as much as endogenous fat synthesis was not
22 taken into account in this calculation. In these conditions, fatty acid catabolism should have
23 occurred in animals. But also, the C16:0 and C18:0 levels - or proportions - in meat and fat of
24 the animals that received CSK increased sharply, indicating a higher lipogenic activity. It is
25 questionable whether poultry degrades preferentially fatty acids belonging to certain families.

1 It is not excluded that the ability of poultry to metabolise n-6 fatty acids is higher than that to
2 metabolise fatty acids from n-9 serie, with emphasis the oleic acid. The hypothesis for
3 efficient n-6 catabolism in poultry is supported by the fact that there is generally a weak
4 relationship between n-6 diet incorporation and levels of n-6 in animal tissues (Lopez-Ferrer
5 et al. 1991; Bavelaar and Beynen 2003).

6 This experiment suggests thus that poultry used unsaturated fatty acids for their energy
7 metabolism and thus spared others nutrients of the diets, such as starch, for lipid synthesis. In
8 such conditions, the resistance of poultry to warm conditions may have been improved with
9 CSK. If this hypothesis was confirmed, the use of CSK could be promoted in poultry feeding
10 under warm conditions.

11 Concerning the chemical fatty acid profiles of adipose and muscle tissue, MUFA and SFA
12 were the main fractions. These results are in agreement with those of Sheu and Chen (2002),
13 De Marchi et al. (2005) and Jahan and Paterson (2007) that observed that C18: 1 n-9, C18: 0
14 and C18: 2 n-6 were, by decreasing order, the most abundant fatty acids in the abdominal
15 muscles of the chickens. In our experiment, the main fatty acids were respectively C18:1 n-9,
16 C16:0, C18:2 n-6 and C18:0, with more than 90% fatty acids.

17 As previously seen, this profile doesn't match that, more unsaturated, that characterizes the fat
18 of groundnut or cotton, or the fat of the diets formulated in this experiment. However, the
19 experimental changes in fatty acids levels or proportions observed in meat and fat are in
20 agreement with those observed in the diets offered, i.e., a decrease in C18:1 n-9 and an
21 increase in C18:2 n-6 with CSK incorporation.

22 Meat fatty acid profile from birds could be easily modified by nutritional manipulations
23 (Chanmugam et al., 1992; Schiavone et al., 2004). O'Keefe et al. (1995) and Jahan et al.
24 (2004) reported that the n-3 fatty acid levels in the pectoral muscles depended of the feed fatty
25 acid profile. Meta-analysis performed by Bavelaar and Beynen (2003) highlighted the

relationships between dietary n-3 and n-6 fatty acids and corresponding levels in meat and fat. Crespo and Esteve-Garcia (2001), as for them, observed that the extents of PUFA changes were more pronounced in meat fat than in abdominal fat. Similar observations were made in this experiment between breast fat and subcutaneous fat, the response of fat tissue in terms of PUFA being not significant.

In conclusion, the substitution of groundnut cake by CSK in iso-nutrient broiler diets had a positive linear effect on diet palatability, animal growth and carcass yield. Changes in dietary fatty acid profiles were observed in subcutaneous fat and in meat fat. These changes suggest a preferential catabolism of a certain type of unsaturated fatty acids for energy production. It appears also that fatty acid changes differed according to the type of body fat. Finally, glandless cotton seeds could be probably considered as a high-quality feed in broiler production, especially when temperature and moisture are high.

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References

- Alford, B.B., G.U. Liepa and A.D. Vanberber, 1996. Cottonseed protein: What does the future hold? *Plant Food Hum. Nutr.*, 49: 1-11.
- Altman, D.W., R.D. Stipanovic and Bell, A.A., 1990. Terpenoids in foliar pigment glands of A, D, and AD genome cottons: Introgression potential for pest resistance. *J. Hered.*, 81: 447-454.
- Amipig, 2000. Digestibilité idéales standardisée des acides aminés des matières premières chez le porc. <http://www.feedbase.com/downloads/amipfra.pdf> (accessed 11th April 2007).
- AOAC, 1990. Official methods of Analysis, 15th edition, Association of Official Analytical Chemists, Washington DC.
- Azman, M.A. and M. Yilmaz, 2005. The growth performance of broiler chicks fed with diets containing CSM supplemented with Lysine. *Rev. Med. Vet.*, 156 (Suppl. 2) : 104-106.
- Bavelaar, F.J. and A.C. Beynen, 2003. Relationships between dietary fatty acid composition and either melting point or fatty acid profile of adipose tissue in broilers. *Meat Sci.*, 64: 133-140.
- Brambila, S. and F.W. Hill, 1966. Comparison of neutral fat and free fatty acids in high lipid-low carbohydrate diets for the growing chicken. *J. Nutr.*, 88: 84-92.
- Chanmugam, P., M. Boudreau, T. Boutte, R.S. Park, J. Hebert, L. Berrio and D.H. Hwang, 1992. Incorporation of different types of n-3 fatty acids into tissue lipids of poultry. *Poult. Sci.*, 71: 516-521.
- Cobb, 2004. Cobb broiler management guide. http://www.cobb-vantress.com/Publications/documents/focus_cobb_1-05.pdf (Accessed 23th february 2007).

1 Cooper, M.A. and K.W. Washburn, 1998. The relationships of body temperature to weight
2 gain, feed consumption and feed utilization in broilers under heat stress. *Poult. Sci.*, 77: 237-
3 242

4 Crespo, N. and E. Esteve-Garcia, 2001. Dietary fatty acid profile modifies abdominal
5 deposition in Broiler chickens. *Poult. Sci.*, 80: 71-78.

6 Dale, N.M. and H.L. Fuller, 1979. Effects of diet composition on feed intake and growth of
7 chicks under heat stress. I. Dietary fat levels. *Poult. Sci.*, 58:1529–1534.

8 De Marchi, M., M. Cassandro, E. Lunardi, G. Baldan and P.B. Siegel, 2005. Carcass
9 characteristics and qualitative meat traits of the Padovana breed of chicken. *Int. J. Poult. Sci.*,
10 4: 233-238.

11 Dersjant-Li, Y., M.W. Verstegen and W.J. Gerrits, 2003. The impact of low concentrations of
12 aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. *Nutr. Res. Rev.*,
13 16: 223-39.

14 Gamboa, D.A., M.C. Calhoun, S.W. Kuhlmann, A.U. Haq and C.A. Bailey, 2001. Use of
15 expander cottonseed meal in broiler diets formulated on a digestible Amino acid Basis. *Poult.*
16 *Sci.*, 80: 789-794.

17 Henry, M.H., G.M. Pesti, R. Bakalli, J. Lee, R.T. Toledo, R.R. Eitenmiller and R.D. Phillips,
18 2001. The performance of Broiler chicks fed diets containing extruded cottonseed meal with
19 Lysine. *Poult. Sci.*, 80: 762 – 768.

20 INRA, 1984. L'alimentation des animaux monogastriques : porcs, lapin, volailles. INRA,
21 Paris.

22 Jahan, K. and A. Paterson, 2007. Lipid composition of retailed organic, free-range and
23 conventional chicken breasts. *Int. J. Food Sci. Technol.*, 42: 251-262.

1 Jahan, K, A. Paterson and C. M. Spickett, 2004. Fatty acid composition, antioxidants and lipid
2 oxidation in chicken breasts from different production regimes. *Int. J. Food Sci. Technol.*, 39:
3 443-453.

4 Lopez-Ferrer, S., M.D. Baucells, A.C. Barroeta and M.A. Grashorn, 2001. n-3 enrichment of
5 chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on
6 meat quality: fish oil. *Poult. Sci.*, 80: 741-752.

7 Lordelo, M.M., A.J. Davis, M.C. Calhoun, M.K. Dowd and N.M. Dale, 2005. Relative
8 toxicity of gossypol enantiomers in broilers. *Poult. Sci.*, 84: 1376-1382.

9 Lusas, E.W. and G.M. Jinidin, 1987. Glandless cottonseed: A review of the first 25 years of
10 processing and utilisation research. *J. Am. Oil Chem. Soc.*, 64: 839-854.

11 Mitchell, A.D., R.W. Rosebrough and J.M. Conway, 1997. Body composition analysis of
12 chickens by dual energy x-ray absorptiometry. *Poult. Sci.*, 76: 1746-1752.

13 Morgan, S.E., E.L. Stair, T.M. Martin, W.C. Edwards and L. Morgan, 1988. Clinical,
14 clinicopathologic, pathologic, and alterations associated with gossypol toxicosis in feeder
15 lambs. *Am. J. Vet. Res.*, 49: 493-499.

16 Nagalakshmi, D., V. Savaram and R. Rama., 2007. Cottonseed meal in poultry diets: A
17 review. *Poult. Sci.*, 44: 119-134.

18 Nahashon, S.N., N. Adefope, A. Amenyenu and D. Wright, 2006. Effect of Varying
19 Metabolizable Energy and Crude Protein Concentrations in Diets of Pearl Gray Guinea Fowl
20 Pullets 1. Growth Performance. *Poult. Sci.*, 85: 1847-1854.

21 NRC, 1994. *Nutrient Requirements of Poultry*, 9th Rev ed., Washington, DC, National
22 Academy Press.

23 O'Keefe, S.F., F.G. Proudfoot and R.G. Ackman, 1995. Lipid oxidation in meats of omega-3
24 fatty acid enriched broiler chickens. *Food Res. Int.*, 28: 417-424.

1 Ojewola, G.S., S.N. Ukachuckwu and E.I. Okulonye, 2006. Cottonseed meal as substitute for
2 soybean meal in broiler ration. *Int. J. Poult. Sci.*, 5: 360-364.

3 SAS Institute, 1999. *Statistics Users Guide, Statistical Analysis System*, 5th edition, 8.2
4 version, Carry, NC, SAS Institute Inc.

5 Schiavone, A., I. Romboli, R. Chiarini and M. Marzoni, 2004. Influence of dietary lipid
6 source and strain on fatty acid composition of Muscovy duck meat. *J. Anim. Physiol. Anim.*
7 *Nutr.*, 88: 88-93.

8 Sheu, K.S. and T.C. Chen, 2002. Yield and quality characteristics of edible broiler skin fat
9 obtained from five rendering methods. *J. Food Engin.*, 55: 263-269.

10 Sukhija, P.S. and D.L. Palmquist, 1988. Rapid method for determination of total fatty acid
11 content and composition of feedstuffs and feces. *J. Agric. Food Chem.*, 36, 1202–1206.

12 Sunilkumar, G., L.M. Campbell, L. Puckhaber, R.D. Stipanovic and K.S. Rathore, 2006.
13 Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic
14 gossypol. *Proc. Nat. Acad. Sci. USA*, 103:18054-18059.

15 Vroh-Bi, I., J.P. Baudoin, B. Hau and G. Mergeai, 1999. Development of high-gossypol
16 cotton plants with low-gossypol seeds using tri-species bridge crosses and in vitro culture of
17 seed embryos. *Euphytica*, 106: 243-251.

18 Watkins, B.A., 1991. Importance of essential fatty acids and their derivatives in poultry. *J.*
19 *Nutr.*, 121: 1475-1485.

20 Willard, S.T., D.A. Neuendorf, A.W. Lewis and R.D. Randel, 1995. Effect of free gossypol in
21 the diet of pregnant and postpartum brahman cows on calf development and cow
22 performance. *J. Anim. Sci.*, 73: 496 – 507.

23 Yo, T., 1991. Utilisation directe des graines de coton décortiquées de variétés sans gossypol
24 dans l'alimentation des poulets de chair en Côte-d'Ivoire. *Rev. Elev. Méd. Vét. Pays Trop.*,
25 44: 355-360.

1 **Table 1.** Chemical composition and amino acid profile of the cottonseed kernels used in the experiment

	Levels (g/kg)
Dry matter	944.5
Ash	117.8
Crude protein	358.9
Ether extract	368.2
Crude fiber	84.0
Asp	32.3
Ala	13.2
Arg	39.8
Cys-Cys	5.7
Glu	64.6
Gly	14.5
His	10.9
Ile	11.5
Leu	21.3
Lys	16.8
Met	5.2
Phe	19.2
Pro	12.9
Ser	15.8
Thr	11.0
Tyr	10.2
Val	16.4

Table 2. Composition of the diets offered to control groups (CG) and of the experimental diets in which groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%.

	CG	G25	G50	G75	CG	G25	G50	G75
	Starting period				Growing period			
Ingredients (g/kg)								
Corn	200.0	250.0	230.0	200.0	420.0	420.0	300.0	270.0
Millet	365.0	325.0	350.0	385.0	210.0	215.0	355.0	390.0
Groundnut cake	250.0	187.5	125.0	62.5	250.0	187.5	125.0	62.5
Cottonseed kernel	0.0	62.5	125.0	187.5	0.0	62.5	125.0	187.5
Fish by-product meal	90.0	100.0	105.0	110.0	27.5	32.5	32.5	37.5
Tricalcium phosphate	12.4	3.0	0.0	0.0	15.0	15.0	5.0	0.0
Groundnut oil	50.0	40.0	30.0	20.0	50.0	40.0	30.0	20.0
L-Lysine HCl	1.6	1.5	2.0	2.0	1.5	1.5	1.5	1.5
DL-Methionine	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin-mineral premix ¹	30.0	30.0	32.0	32.0	25.0	25.0	25.0	30.0
Chemical composition (g/kg)								
Dry matter	933.0	934.0	938.0	938.0	937.0	929.0	933.0	932.0
Crude protein	205.0	216.0	222.0	210.0	195.0	197.0	184.0	182.0
Ether extract	121.7	113.9	118.3	123.7	99.8	100.0	112.7	113.9
Crude fiber	25.0	22.0	27.0	28.0	27.0	30.0	31.0	27.0
Ash	63.0	84.0	81.0	81.0	76.0	66.0	67.0	68.0
Ca	11.0	13.0	12.0	12.0	10.0	8.0	9.0	12.0
Total P	9.0	9.0	9.0	10.0	8.0	7.0	8.0	8.0
K	6.0	6.0	6.0	5.0	6.0	6.0	6.0	6.0
Na	1.0	2.0	2.0	1.0	1.0	1.0	1.0	1.0
Mg	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Others								
ME, Kcal/kg	3598	3518	3540	3559	3463	3418	3496	3521
Total gossypol, mg/kg	0.0	29.1	49.5	85.4	0.0	23.4	46.4	67.1
Free gossypol, mg/kg	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

¹Macro-vetamix 5% (Vetagropharma technology) which provided (per kg of premix): Ca, 280 g; P, 37g; NaCl, 33g; Mn, 1.4 mg; Zn, 1.2 mg; Fe, 1.4 mg; Cu 0.2 mg; I, 8 mg; Co, 2 mg; Se, 2.8 mg; vitamin A, 250,000 IU; vitamin D₃, 50,000 IU; vitamin E, 290 mg; vitamin B1, 55 mg; vitamin B2, 100 mg; vitamin B3, 480 mg; vitamin B5, 195 mg; vitamin B6, 55 mg; vitamin B12, 600 µg; vitamin K3, 50 mg; folic acid vitamin, 27 mg; vitamin C, 175 mg; H biotin vitamin, 600 µg; Lysine HCl, 5%; Methionine, 3%

1 **Table 3.** Fatty acid profile of feeds offered to broilers that received either a control diet (CG) or diets in which
2 groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%

	Starting diet				Growing diet			
	CG	G25	G50	G75	CG	G25	G50	G75
<i>Fatty acids (g/kg fat)</i>								
C14 :0	7	8	9	10	5	6	5	6
C16 :0	132	136	140	152	124	132	124	131
C18 :0	43	39	36	34	34	30	33	31
C20 :0	10	8	6	5	9	6	8	6
C16:1n7	8	10	11	12	6	7	5	7
C18:1n9/7	490	460	397	337	470	346	375	319
C20:1n9	9	7	5	4	7	4	5	4
C18:2n6	282	318	382	434	329	457	433	485
C20:4n6	5	4	3	3	5	3	3	2
C18:3n3	11	8	8	7	8	7	9	8
C20:5n3	3	3	3	3	3	1	0	1
SFA	191	191	191	201	172	175	169	174
MUFA	507	476	412	352	483	356	386	329
W6	287	321	385	437	334	461	435	487
W3	14	11	11	10	11	8	10	9
PUFA	301	333	396	446	345	469	445	496

Table 4. *Animal performance of Cobb 500 broilers that received either a control diet (CG) or diets in which groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%*

	CG	G25	G50	G75	SEM	P>F
Initial weight, g	43	43	41	42	0.43	0.426
Weight at d22, g	353a	415b	550c	593d	10.4	0.001
Final weight, g	1063a	1262b	1635c	1847d	42.3	0.001
ADG starting period, g/d	14.8	17.7	24.2	26.2	-	-
ADG growing period, g/d	33.8	40.3	51.7	59.7	-	-
Feed intake starting period, g	24.9	28.9	41.1	42.4	-	-
Feed intake growing period, g	88.7	95.9	125.0	129.8	-	-
Feed conversion ratio starting period	1.54	1.39	1.64	1.53	-	-
Feed conversion ratio growing period	2.72	2.60	2.67	2.39	-	-

Values on the same line with no common letter are significantly different at $P < 0.05$.

1 Table 5. *Nutrients digestibility (%) of Cobb 500 broilers that received either a control diet (CG) or diets in*
2 *which groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%*

Items	CG	G25	G50	G75	SEM	P>F
<i>Starting diets</i>						
Dry matter	65.14	64.27	65.45	65.59	1.9	0.96
Organic matter	72.47	71.54	73.03	73.48	1.6	0.84
Fat matter	67.37	66.98	68.80	69.43	1.7	0.73
<i>Growing diets</i>						
Dry matter	66.12	66.33	67.75	68.80	2.0	0.78
Organic matter	72.58	72.74	74.04	74.21	1.8	0.88
Fat matter	70.68	70.51	69.07	71.40	1.8	0.82

3 The values on the same line with no common letter are significantly different at $P < 0.05$

1 **Table 6.** *Animal performance and carcass characteristics of broilers that received either a control diet (CG) or*
2 *diets in which groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75*
3 *(G75)%*

	CG	G25	G50	G75	SEM	P>F
<i>Weights, g</i>						
Live weight	1040a	1435b	1672c	1930d	139.4	0.062
Carcass weight	717a	1006b	1229c	1379d	113.4	0.041
Breast	245a	363b	450c	560d	28.8	0.001
Thigh	337a	435b	495b	573c	42.0	0.424
Wing	113a	157bc	172c	208d	11.6	0.059
Liver	53	52	54	52	4.6	0.988
Heart	12	12	12	13	1.7	0.948
Gizzard	41	49	41	47	5.8	0.690
<i>Proportions (%)</i>						
Dressing	68.90a	70.10bc	73.50c	71.45b	0.9	0.012
Breast/carcass	34.17a	36.08a	36.61ab	40.61b	1.1	0.016
Thigh/carcass	47.00	43.24	40.28	41.55	1.6	0.560
Wing/carcass	15.76	15.61	13.99	15.08	0.5	0.762

4 On a line, means with no common letter are significantly different at $P > 0.05$

Table 7. Ether extract and fatty acids levels (mg/kg DM) in muscle of broilers that received either a control diet (CG) or diets in which groundnut cake was substituted by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%

	CG	G25	G50	G75	P>F	SEM
<i>Ether extract (g/kg DM)</i>	62.7	72.4	66.9	77.2	0.963	21.0
<i>Fatty acids (mg/100g DM)</i>						
C14 :0	29.7a	26.3a	47.7b	49.0b	0.005	4.5
C16 :0	1234.6a	1190.1a	1715.0b	1827.6b	0.001	105.7
C18 :0	626.0a	652.5a	911.0b	963.9b	0.007	69.3
C16:1 n-9	136.8	87.8	88.4	81.1	0.499	27.9
C18:1 n-9	3223.3a	3251.1a	2065.3b	1847.8b	0.002	247.8
C18:2 n-6	1043.2a	1083.7a	1452.0b	1524.3b	0.015	108.4
C18:3 n-6	5.3a	5.1a	7.0ab	7.9a	0.048	0.7
C20:3 n-6	27.1	28.0	29.6	26.1	0.624	1.8
C20:4 n-6	151.0	149.8	135.3	141.7	0.726	10.5
C22:4 n-6	32.6	31.3	31.9	28.4	0.661	2.5
C18:3 n-3	18.7a	19.3a	24.2b	23.4b	0.004	1.0
C20:5 n-3	7.2	5.4	7.0	5.8	0.469	0.9
C22:5 n-3	31.7	31.2	34.3	28.2	0.510	2.6
C22:6 n-3	27.7	31.2	28.1	30.1	0.892	3.6
Other SFA	8.8a	10.3a	11.9ab	14.3b	0.079	1.4
Other MUFA	25.7a	24.6a	28.7b	25.1a	0.019	0.8
Other PUFA	26.1	27.4	33.9	27.5	0.193	2.4
SFA	1910.5a	1890.9a	2701.2b	2869.5b	0.002	177.8
MUFA	3385.8a	3363.5a	2182.5b	1954.0b	0.003	267.6
Total n-6	1278.0a	1319.1a	1683.5b	1751.8b	0.016	108.1
Total n-3	92.5	93.3	99.6	91.5	0.875	7.0
PUFA	1370.6a	1412.4a	1783.1b	1843.3b	0.015	107.7
MUFA/SFA	1.8a	1.6a	0.8b	0.7b	<.0001	0.10
PUFA/SFA	0.8	0.8	0.7	0.7	0.360	0.04
PUFA/MUFA	0.4a	0.5a	0.9b	1.0b	<.0001	0.05
UFA/SFA	2.6a	2.4a	1.5b	1.3b	<.0001	0.11
n-6/n-3	13.3a	14.2a	16.1a	17.6b	0.119	1.2

On a line, means with no common letter are significantly different at $P > 0.05$

1 Table 8 *Fatty acids proportions in the subcutaneous fat and muscles fat of broilers that received either a control diet (CG) or diets in which groundnut cake was substituted*
2 *by cottonseed kernels at levels of 25 (G25), 50 (G50) or 75 (G75)%*

Fatty acid (% fat)	Sub-cutaneous fat				Meat fat				P>F			SEM
	CG	G25	G50	G75	CG	G25	G50	G75	Loc.	Group	Inter.	
C14:0	0.65a	0.66ab	0.77b	0.8b	0.39a	0.40a	0.66b	0.67b	0.001	0.001	0.164	0.041
C16:0	22.91a	23.94a	26.73b	27.32b	17.65a	18.34a	25.30b	26.86b	0.001	0.001	0.002	0.721
C18:0	8.85a	10.47a	12.5b	13.43b	9.79a	10.45a	14.07b	14.49b	0.057	0.001	0.643	0.624
C16:1 n-9	2.94	2.01	1.67	1.42	1.54	1.12	1.13	1.15	0.017	0.143	0.600	0.426
C18:1 n-9	40.89a	37.57ab	33.06ab	30.66b	47.94a	46.47a	29.06b	28.1b	0.070	0.001	0.001	1.740
C18:2 n-6	21.7	23.26	23.39	24.49	15.98a	16.85a	22.43b	22.08b	<0.001	0.002	0.073	1.148
C18:3 n-6	0.13	0.13	0.13	0.14	0.07a	0.08ab	0.11b	0.10ab	<0.001	0.150	0.546	0.011
C20:3 n-6	0.07	0.08	0.07	0.08	0.50	0.47	0.60	0.50	<0.001	0.861	0.820	0.076
C20:4 n-6	0.08	0.09	0.07	0.09	2.83	2.69	2.81	2.70	<0.001	0.997	0.996	0.399
C22 :4 n-6	0.02	0.03	0.02	0.02	0.61	0.56	0.63	0.55	<0.001	0.951	0.925	0.080
C18:3 n-3	0.56a	0.53a	0.51ab	0.47b	0.26a	0.27a	0.35b	0.32ab	<0.001	0.379	0.002	0.021
C20:5 n-3	0.02	0.02	0.02	0.01	0.13	0.09	0.15	0.12	<0.001	0.524	0.646	0.024
C22:5 n-3	0.02	0.02	0.02	0.02	0.60	0.54	0.69	0.53	<0.001	0.804	0.809	0.091
C22:6 n-3	0.01	0.01	0.02	0.01	0.54	0.52	0.6	0.57	<0.001	0.967	0.971	0.085
Other SFA	0.43	0.49	0.41	0.47	0.32a	0.33ab	0.41b	0.42b	<0.001	0.148	0.065	0.030
Other mufa	0.59a	0.55a	0.46b	0.42b	0.37	0.36	0.40	0.36	<0.001	0.015	0.003	0.024
Other pufa	0.12	0.14	0.15	0.16	0.48	0.47	0.60	0.50	<0.001	0.640	0.730	0.059
SFA	32.84a	35.56a	40.41b	42.02b	28.14a	29.52a	40.43b	42.44b	<0.001	0.005	0.021	1.179
MUFA	44.41a	40.13ab	35.2b	32.5b	49.85a	47.95a	30.59b	29.62b	0.338	<0.001	0.012	2.064
Total n-6	22.12	23.73	23.82	24.97	20.37a	21.01a	27.08b	26.35b	0.971	<0.007	0.146	1.404
Total n-3	0.62	0.58	0.57	0.51	1.65	1.53	1.89	1.6	<0.001	0.782	0.785	0.194
PUFA	22.75	24.31	24.39	25.48	22.01a	22.54a	28.97b	27.95b	0.306	0.013	0.161	1.520
MUFA/SFA	1.37a	1.15a	0.89b	0.78b	1.79a	1.65a	0.76b	0.72b	<0.023	<0.001	0.008	0.025
PUFA/SFA	0.69	0.69	0.61	0.61	0.78	0.76	0.72	0.66	0.016	0.102	0.936	0.045
PUFA/MUFA	0.53a	0.62ab	0.70ab	0.79b	0.45a	0.48a	0.96b	0.96b	0.289	<0.001	0.017	0.011
UFA/SFA	2.06a	1.83a	1.49b	1.38b	2.57a	2.42a	1.48b	1.36b	0.002	<0.001	<0.001	0.025
n-6/n-3	35.46a	40.52ab	41.82ab	49.01b	12.68	14.44	15.95	18.72	<0.001	<0.001	0.347	1.975

3 On a line and in a location, means with no common letter are significantly different at P > 0.05