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Nutritional composition, antioxidant activity and gossypol level of Nazilli glandless cottonseed, cottonseed kernel and their cold-pressed meal

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Abstract

Background The study was conducted to determine the nutrient composition and gossypol levels of glandless cottonseed (GCS), glandless cottonseed kernel (GCSK) glandless cottonseed meal (GCSM) and cottonseed kernel meal (GCSKM) obtained by cold pressing of cotton seeds and kernels (Glandless Nazilli variety) as a raw material of compound feed industry.

Result Feed and dry matter (DM)-based nutrient analyses showed significant differences ($p < 0.05$) in DM, OM (organic matter), CP (crude protein), EE (ether extract), ash and NFE (nitrogen-free extract) contents between GCS and GCSK. The DM-based K, P, S, Mg, Ca, Na, Fe, Cu, Zn and Al contents were significantly different ($p < 0.05$) between GCS and GCSK. The concentrations of nonessential heavy metals (Cd, Pb, Ni and Al) in GCS, GCSK, GCSM and GCSKM samples were below the permissible limits. The linoleic acid (C18:2; ω6) was the main component (55.55%) among the 20 fatty acids identified in GCS oil. The level of unsaturated fatty acids (70.78%) was higher than that of saturated fatty acids (29.22%). Total phenolics concentrations of GCS, GCSK, GCSM and GCSKM samples were 7.87, 2.18, 5.86 and 1.91 mg gallic acid equivalents (GAE)/g, respectively. Free and total gossypol levels of GCS and GCSK were 294 and 440, and 521 and 706 mg/kg, respectively.

Conclusion The results revealed that nutritional properties of Nazilli GCS with low gossypol and high linoleic acid content were relatively higher compared to the other meals investigated. The antioxidant activity of phenolic compounds, albeit at low levels in Nazilli GCS and GCSM, may contribute to animal health and production efficiency when used in animal rations.

Keywords Glandless cottonseed, Nutrient composition, Mineral, Gossypol, Fatty acids, Antioxidant activity

1 Background

Cottonseed meal is the by-product of oil extraction from cotton seeds. Cottonseed meal is a common source of protein for ruminants and poultry. It is an attractive promising plant protein substitute with 22–56% crude protein (CP) and 7.4–11.99 MJ/kg of metabolizable energy because it is more economical than soybean meal and can provide abundant protein to meet the requirements for animals. Cotton by-products are potentially viable options in diet formulation due to their chemical

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composition and low cost. Previous studies indicated that cottonseed meal can be included at low levels as a protein source in livestock feed, while higher rates may lead to a significant decrease in growth performance and cause mortality due to the presence of gossypol [1–3]. Gossypol is a polyphenolic compound found in the secretory glands of cotyledons in cottonseed freely or bounded to other substances. The American Oil Chemists Society [4] stated that the gossypol and its derivatives, which can be extracted using 70% aqueous acetone, are known as free gossypol. The gossypol is bounded when cottonseed is processed with heat, moisture and pressure, where gossypol reacts with the epsilon group of amino acids, especially lysine and arginine. The free gossypol has a toxic effect due to the 15 toxic glycoside compounds in the root and meal, mainly in the seed. The gossypol concentration in the seed or meal as well as protein and fat levels varies depending on the type of plant, the soil in which the plant grows, oil extraction method, linter, seed coat and climate [5, 6]. The cheapest way to eliminate the harmful effects of gossypol on human and animal nutrition is to obtain new hybrids by breeding varieties that do not contain gossypol in their seeds. Glandless cotton seed (GCS) obtained by breeding in Aydın/Nazilli Cotton Research Institute (1991–2001) was registered in 2002. The Nazilli GCS is a productive and has superior technological values. The use of cottonseed and meal in the feeding of various farm animals has been extensively studied, however, very few studies have been reported on GCS and GCSM in the literature. Therefore, the objective of this study was to determine the nutrient contents of recently bred Nazilli GCS variety, and to reveal the mineral compositions, total phenolic compound, antioxidant capacity and gossypol levels of Nazilli GCS.

2 Methods

Feed materials of the experiment were Nazilli Glandless GCS–GCSK, cold-pressed GCSM–GCSKM raw materials produced in the Ministry of Agriculture and Forestry Nazilli/Aydın Cotton Research Institute Directorate of Türkiye. The cottonseeds were first delinted in the cottonseed processing plant located at the Cotton Research Institute. The shell was physically cracked in the laboratory to exacinate the kernel from the cottonseed, and the kernel was manually removed. Cottonseed oil was expelled using a cold press (BNK SP1560d, Ankara, Türkiye) machine. The cold press machine was set to a 5-mm-diameter outlet tip, 20 rpm screw rotation speed and a constant outlet temperature of 40 °C. The GCSM and GCSKM were obtained separately from GCS and GCSK and labeled. The GCS was used for cotton oil production, and the oil was filtered through a filter (Miroil) to remove the solid impurities and stored in a clean vial tube. Two different GCSM and GCSKM taken from the cold press machine were passed through the blender, and the samples were taken for basic analysis (Fig. 1).

2.1 Analytical methods and nutrient analysis

The samples were grounded in a mill to pass through a 1-mm sieve and prepared for analysis. Chemical analyses were repeated three times in the feed analysis laboratory of a special feed plant. Dry matter (DM), crude protein (CP, using the macro-Kjeldahl method), crude fiber (CF), ether extract (EE), ash and organic matter (OM) of the samples were analyzed in accordance with AOAC [7]. The nitrogen-free extract (NFE) was determined using the following equation (Eq. 1):

$$\mathrm{NFE} = \left(\left(\text{organic matter (OM)} - (\mathrm{EE} + \mathrm{CP} + \mathrm{CF})\right)\right) \quad (1)$$

where the NFE is the nitrogen-free extract, OM is the organic matter, EE is the ether extract, CP is the crude protein, and CF is the crude fiber content.

The metabolic energy values were calculated using the results of nutrient analysis. The equation defined by TSE [8] was used to calculate the metabolic energy values as follows (Eq. 2):

$$\mathrm{ME}\ (\mathrm{kcal/kg}) = 3260 + [0.455 \times \mathrm{CP\%}] + [3.517 \times \mathrm{EE\%}] - [4.037 \times \mathrm{CF\%}] \quad (2)$$

where CP is the crude protein, EE is the ether extract, and CF is the crude fiber content.

2.2 Mineral analysis

The samples were dried in an oven at 70 °C for 48 h and 0.2 g was weighed to determine the mineral concentration of each sample. Then, 5 mL of HNO_3 and 2 mL of H_2O_2 were added to these samples and burned in a microwave device (Mars 6). The final volume was filled to 20 mL with distilled water and the concentrations of Ca, P, K, S, Na, Mg, Al, Zn, Fe, Mn, Cu, B, Ni and Cd were determined using an ICP-OES (Varian vista) device [9].

2.3 Preparation of extracts for antioxidant activity tests

Two-hundred mg ground sample (cottonseed, cottonseed kernel and their cold-pressed meal) was weighed into a glass tube and 10 mL of methanol/dichloromethane mixture (5:1) was added to the tube. Following the vortex, the samples were kept in an ultrasonic bath for 30 min. The resulting extraction solutions were removed by a rotary evaporator and stock solutions were prepared at a ratio of 2 mg/mL. The stock solutions were stored at 4 °C for antioxidant activity tests and total phenolic content analysis. The antioxidant activity analyses were performed

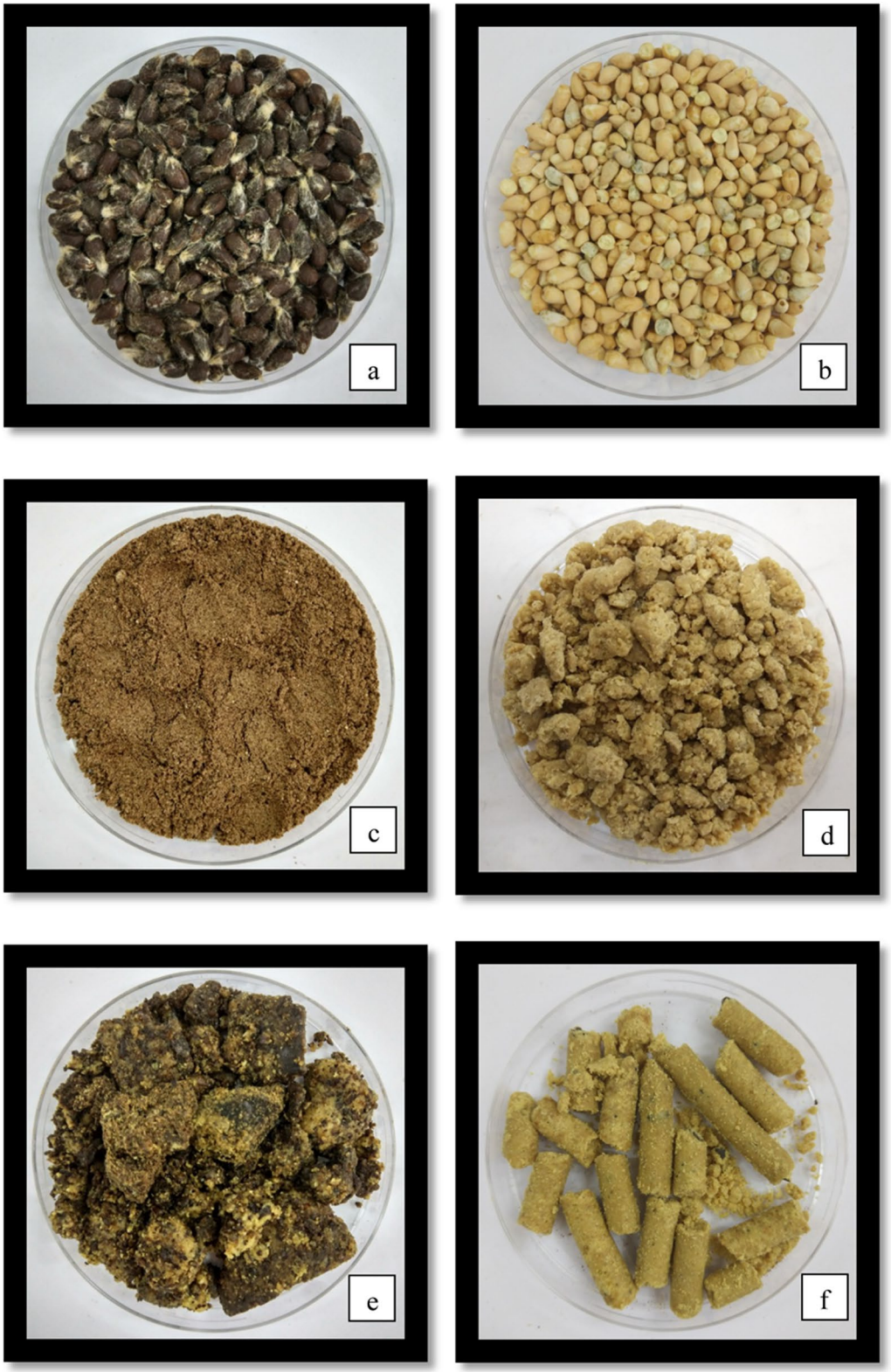

Fig. 1 **a** Glandless cottonseed, **b** Glandless cottonseed kernel, **c** Ground glandless cottonseed, **d** Ground glandless cottonseed kernel, **e** Glandless cottonseed meal, **f** Glandless cottonseed kernel meal

in 3 repetitions, and the results were reported with the mean and standard error values of 3 replicates.

2.4 Total phenolic content

Total phenolic compound content was determined using the Folin–Ciocalteu reagent [10]. An amount of 0.2 mL was taken from stock solutions prepared with cottonseed extracts and topped up to 4.6 mL with distilled water. The mixture was placed on a vortex after the addition of 0.3 mL Na_2CO_3 solution (2%) and 0.1 mL Folin–Ciocalteu reagent. The solution was kept in room conditions for 2 h and the absorbance was measured using a spectrometer at 760 nm. The results were calculated as the amount of gallic acid equivalent matter (mg GAE/g).

2.5 Cation radical scavenging activity (TEAC)

The TEAC value is the ability of antioxidant to scavenge the radical cation (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) measured by spectrophotometric analysis [11]. The formation of $ABTS^{\cdot+}$ was directly produced by potassium persulfate ($K_2S_2O_8$) oxidation of $ABTS^{\cdot+}$. For this process, a 2 mM $ABTS^{\cdot+}$ solution was prepared. $ABTS^{\cdot+}$ radical was obtained by adding 2.45 mM potassium persulfate solution to this solution. For each sample (10, 20, 30 and 40 µL (mg/mL)), four different amounts of samples were prepared in triplicate. The prepared samples were added to phosphate buffer (0.1 M, pH = 7.4) in a volume of 3 mL. $ABTS^{\cdot+}$ solution (1 mL) was then added to all samples and their absorbance was measured at 734 nm. The results were expressed as trolox equivalent antioxidant capacity (TEAC) µmol/g extract.

2.6 Iron reducing power activity (FRAP)

Total antioxidant activity was determined using the FRAP assay by the method of Oyaizu [12]. The reducing agent in the medium reduces Fe^{3+} ions to Fe^{2+} ions and the absorbance of the Prussian blue complex formed by the addition of $FeCl_3$ is measured. A high absorbance value indicates a high reducing capacity. For each sample (10, 20, 30 and 40 µL (mg/mL)), four different amounts were taken in triplicate and the volume was made up to 1.25 mL with phosphate buffer (0.2 M, pH = 6.6) and 1.25 mL of potassium ferric cyanide [$K_3Fe(CN)_6$] (1%) was added. After incubation at 50 °C for 20 min, the reaction mixture was brought to room temperature, 1 mL of 10% TCA and 250 µL of 0.1% $FeCl_3$ were added, and absorbance values were measured at 700 nm. The results are given as absorbance values at 700 nm versus the concentration of the samples. The FRAP results were calculated as the amount of Trolox equivalent matter (TE) µmol/g extract.

2.7 Free radical scavenging activity DPPH (1,1-diphenyl-2-picrylhydrazyl) test

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging capacity in cottonseed extracts was determined following the procedure described by Blois [13]. The extract solutions in different quantities were placed in the test tubes and their volumes were completed to 3 mL with ethyl alcohol. DPPH solution (1 mL, 0.26 mM) was added to them and mixed by means of a vortex. After having waited for 30 min in the dark, their absorbances were read on a spectrophotometer at 517 nm. The results were expressed as µmol trolox equivalent (TE)/g extract.

2.8 Cupric ion reducing antioxidant capacity (CUPRAC)

The CUPRAC was determined using the method reported by Apak et al. [14]. Each extract (40–320 µg/mL, 1 mL) was mixed with a solution of $CuCl_2$ (1 mL 1.0×10^{-2} M), 1 mL of neocuproine ethanolic solution (7.5×10^{-3} M) and 1 mL of NH_4Ac (1 M) buffer solution were added to a test tube. Absorbance against a reagent blank was measured at 450 nm after 30 min. The CUPRAC values were expressed as µmol Trolox equivalent per gram of extract.

2.9 Free and total gossypol analysis

Free and total gossypol levels of GCS and GCK were determined spectrophotometrically using the TS 5889 method of Turkish Standards Institute [15]. Free gossypol in GCS and GCK was extracted using a mixture of 3-amino-1-propanol, isopropanol hexane and glacial acetic acid. Total gossypol was extracted using a solution of 3-amino-1-propanol, dimethyl form amide and glacial acetic acid. The gossypol was converted to gossypol dianilide with aniline substance. Absorbance at 435 nm was measured by a spectrophotometer (Beckman).

2.10 Fatty acid composition of glandless cottonseed oil by GC-FID

Fatty acid analysis, cold extraction, methyl esterification step and injection process of the crude oil obtained from the cold press machine of glandless cottonseed were carried out in the laboratories. A 50-mg GCS oil sample was dissolved in 3 mL n-hexane in a 20-mL test tube, and 3 mL of 2 N potassium hydroxide in methanol was added. The prepared solution was vortexed for 60 s and centrifuged for 15 min. The supernatant was taken and 1 µL was injected into GC. Fatty acids were analyzed by Perkin Elmer Clarus 500 Chromatography; FID (flame ionization detector) and Restek (Rtx-2330) capillary column

(30 m × 0.25 mm × 0.2 μm) were used. The operating condition of the GC was as follows: helium flow 1 mL/min; flame ionization detector (FID) at 250 °C; splitless injector at 250 °C. The starting temperature of the oven was 120 °C and kept at this temperature for 2 min and increased by 2 °C per minute till reaching 180 °C. The temperature was increased by 4 °C per minute to 200 °C and then left to stand for 3 min at this temperature. A mixture of methyl esters of 37 fatty acids was used as the standard in the identification of fatty acids (Food Industry FAME Mix-Restek). The fatty acid composition was expressed as a percent amount (%) of fatty acid per total fatty acids.

2.10.1 Statistical analysis

Differences in nutrient value between GCS and GCK; and cold-pressed GCSM-GCKM samples were determined by *t* test using SPSS 17 package program [16].

3 Results

3.1 Nutrient composition

Nutrient compositions of glandless cottonseed, cottonseed kernel and their cold-pressed meal in dry matter and feed are presented in Tables 1 and 2, respectively. The DM ($p < 0.01$), OM ($p < 0.05$), CP ($p < 0.01$), EE ($p < 0.05$), ash ($p < 0.01$), NFE ($p < 0.05$) contents in feed and dry matter between GCS and GCSK were significantly different, while the difference in ME was not different. The values of DM, OM, CP, EE and ash in GCSK were higher than those in GCS, except for CF and NFE. High cellulose content of cottonseed shell confirmed higher values of CP and EE in the GCSK. The ME value was not significantly different between the GCS and GCSK. The DM, OM and ME values of cold-pressed cottonseed meal were not significantly different between the GCSM and GCSKM samples. The values of CP ($p < 0.01$), EE ($p < 0.05$) and ash ($p < 0.05$) in GCSKM were higher than those in GCSM. The CF ($p < 0.05$) and NFE ($p < 0.01$) contents of the GCSM samples were higher than those in GCSKM.

Table 1 Nutrient composition of glandless cottonseed, cottonseed kernel and their cold-pressed meal (on % feed basis; Mean ± SEM)

Nutrients	Seed		Meal	
	GCS	GCSK	GCSM	GCSKM
DM	94.79 ± 0.05^{B}	96.65 ± 0.04^{A}	94.77 ± 0.04	94.99 ± 0.05
OM	90.88 ± 0.04^{b}	92.06 ± 0.01^{a}	90.37 ± 0.04	90.22 ± 0.03
CP	25.34 ± 0.40^{B}	38.70 ± 0.17^{A}	31.51 ± 0.06^{B}	39.98 ± 0.01^{A}
EE	22.04 ± 0.08^{b}	35.89 ± 0.51^{a}	24.06 ± 0.15^{b}	30.74 ± 0.42^{a}
CF	16.74 ± 0.88^{a}	2.20 ± 0.18^{b}	13.22 ± 0.21^{a}	7.39 ± 0.37^{b}
Ash	3.92 ± 0.15^{B}	4.59 ± 0.30^{A}	4.41 ± 0.01^{b}	4.77 ± 0.02^{a}
NFE	26.77 ± 0.37^{a}	15.27 ± 0.84^{b}	21.59 ± 0.08^{A}	12.12 ± 0.08^{B}
ME, kcal/kg	3260.2 ± 0.04	3261.3 ± 0.01	3260.5 ± 0.01	3261.0 ± 0.03

DM Dry matter, *OM* Organic matter, *CP* Crude protein, *EE* Ether extract, *CF* Crude fiber, *NFE* Nitrogen-free extract, *ME* Metabolic energy, *GCS* Glandless cottonseed, *GCSK* Glandless cottonseed kernel, *GCSM* Glandless cottonseed meal, *GCSKM* Glandless cottonseed kernel meal

Means within a row followed by different superscripts differ significantly (^{A,B}: $p < 0.01$; ^{a,b}: $p < 0.05$)

Table 2 Nutrient composition of glandless cottonseed, cottonseed kernel and their cold-pressed meal (on % dry matter basis; Mean ± SEM)

Nutrients	Seed		Meal	
	GCS	GCSK	GCSM	GCSKM
OM	95.87 ± 0.04^{a}	95.25 ± 0.01^{b}	95.35 ± 0.04	94.98 ± 0.03
CP	26.73 ± 0.43^{b}	40.05 ± 0.22^{a}	33.24 ± 0.04^{B}	42.09 ± 0.02^{A}
EE	$23.25 \pm 0{,}10^{b}$	$37.13 \pm 0{,}51^{a}$	25.39 ± 0.17^{b}	32.36 ± 0.46^{a}
CF	17.65 ± 0.91^{a}	2.28 ± 0.19^{b}	13.94 ± 0.21^{a}	7.78 ± 0.39^{b}
Ash	4.13 ± 0.02^{b}	4.75 ± 0.03^{a}	4.65 ± 0.01^{b}	5.02 ± 0.01^{a}
NFE	23.03 ± 0.35^{a}	12.45 ± 0.84^{b}	17.55 ± 0.05^{A}	7.74 ± 0.12^{B}

OM Organic matter, *CP* Crude protein, *EE* Ether extract, *CF* Crude fiber, *NFE* Nitrogen-free extract, *GCS* Glandless cottonseed, *GCSK* Glandless cottonseed kernel, *GCSM* Glandless cottonseed meal, *GCSKM* Glandless cottonseed kernel meal

Means within a row followed by different superscripts differ significantly (^{A,B}: $p < 0.01$; ^{a,b}: $p < 0.05$)

3.2 Mineral contents

Mineral compositions of glandless cottonseed, cottonseed kernel and their cold-pressed meal on dry matter basis are presented in Table 3. Dry matter-based mineral contents between GCS and GCSK were significantly different except Mn, Mo, Ni, Cd, Pb and B. The P, S, Mg, Fe, Cu and Zn contents in GCSK were higher than those in GCS. Likewise, the Fe concentration determined in GCSM and GCSKM based on DM was significantly ($p < 0.01$) higher than the concentrations in GCS and GCSK. The excessive level of Fe in GCSM and GCSKM samples indicated a possible Fe contamination in the cold press machine during pressing.

3.3 Total phenolic compound and antioxidant activity

The results of the total phenolic content and antioxidant activity in GCS, GCSK and their cold-pressed meal samples are presented in Table 4. Antioxidants are responsible for preventing oxidative damage to cellular components as a result of biochemical reactions. Total content of phenolic substances, ABTS cation radical scavenging activity, ferric ion reducing antioxidant power, DPPH radical scavenging activities and copper

Table 3 Minerals composition of glandless cottonseed, cottonseed kernel and their cold-pressed meal (on % dry matter basis; Mean ± SEM)

Minerals	Seed		Meal	
	GCS	GCSK	GCSM	GCSKM
K, %	1.18 ± 0.01^{a}	1.09 ± 0.00^{b}	1.27 ± 0.02^{a}	1.11 ± 0.00^{b}
P, %	$0.66 \pm 0{,}01^{B}$	1.01 ± 0.01^{A}	0.87 ± 0.02^{b}	1.00 ± 0.00^{a}
S, %	0.30 ± 0.01^{B}	0.42 ± 0.01^{A}	0.38 ± 0.02	$0.43 \pm 0{,}01$
Mg, %	0.37 ± 0.00^{B}	0.47 ± 0.00^{A}	0.46 ± 0.01	0.47 ± 0.01
Ca, %	0.13 ± 0.00^{A}	0.11 ± 0.00^{B}	0.15 ± 0.00^{a}	0.12 ± 0.00^{b}
Na, mg/kg	54.9 ± 2.05^{A}	27.4 ± 0.50^{B}	45.3 ± 0.00^{a}	24.8 ± 0.50^{b}
Fe, mg/kg	54.9 ± 0.50^{B}	64.2 ± 0.50^{A}	198.9 ± 5.70^{A}	87.9 ± 5.85^{B}
Cu, mg/kg	11.1 ± 0.50^{b}	15.5 ± 0.00^{a}	14.3 ± 0.55	15.8 ± 0.00
Zn, mg/kg	37.5 ± 2.65^{b}	50.2 ± 0.55^{a}	85.5 ± 5.25^{a}	52.6 ± 0.00^{b}
Mn, mg/kg	12.7 ± 0.00	11.4 ± 0.00	14.8 ± 0.00	11.6 ± 0.00
Mo, mg/kg	1.32 ± 0.05	1.45 ± 0.00	1.58 ± 0.00	1.53 ± 0.06
Ni, mg/kg	2.38 ± 0.05	3.05 ± 0.16	3.48 ± 0.21	3.79 ± 0.21
Cd, mg/kg	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Pb, mg/kg	0.13 ± 0.06	0.13 ± 0.07	1.07 ± 0.11	1.33 ± 0.07
B, mg/kg	14.3 ± 0.55	11.9 ± 0.50	15.9 ± 1.05	12.6 ± 0.00
Al, mg/kg	4.2 ± 0.00^{a}	1.6 ± 0.73^{b}	12.7 ± 1.05	14.8 ± 2.15

GCS Glandless cottonseed, *GCSK* Glandless cottonseed kernel, *GCSM* Glandless cottonseed meal, *GCSKM* Glandless cottonseed kernel meal

Means within a row followed by different superscripts differ significantly (^{A,B}: $p < 0.01$; ^{a,b}: $p < 0.05$)

reducing power in seeds and kernels, in shell seed and meal were significantly different ($p < 0.01$).

3.4 Free and total gossypol levels

The results of free and total gossypol levels in GCS and GCSK samples are presented in Table 5. The reason for the high levels of total gossypol was that the embryo parts of the cottonseed, which ensures the high CP content of these groups, found more and the embryo parts contain high levels of gossypol. The gossypol level of GCSK was very high compared to the GCS ($p < 0.01$).

3.5 Fatty acid content of crude GCS oil

The fatty acid composition of the raw cottonseed oil obtained by cold pressing of GCS is given in Table 6.

4 Discussion

4.1 Nutrient composition

The nutrient contents of GCS and GCSK were similar to the values reported by Calhoun et al. [17]. The GCS nutrient contents of current study were higher than the values (90.1% DM; 23.5% CP; 19.3% EE; 4.2% ash) reported by NRC [18]**.** Several researchers [19–24] indicated that the CP ratio of cottonseed meal varies between 23.76 and 50.67%. Ustaoğlu [25] reported that the differences in nutrient contents of cottonseed and cottonseed meal produced different cultivars grown in the central district of Hatay province of Türkiye were significantly different due to the differences in genetic structures of the cultivars. Although the oil and protein content of cottonseed change with genetic variations, they were usually reported between 17–27% EE and 12–32% CP [26]. In addition to genetic variation, environmental conditions and agricultural practices may have significant impact on cottonseed composition [27]. Higher EE content of GCSM and GCSKM (25.39% and 32.36%) reported in the literature may be related to the fact that the oil in the cold press machine is not removed at full capacity due to the contact with the meal.

Table 5 Free and total gossypol levels of glandless cotton seed used in the study (mg/kg; Mean ± SEM)

Gossypol	Glandless Cottonseed	
	GCS	GCSK
Free gossypol	294 ± 15^{B}	521 ± 2^{A}
Total gossypol	440 ± 4^{B}	706 ± 20^{A}

GCS Glandless cottonseed, *GCSK* Glandless cottonseed kernel

Means within a row followed by different superscripts differ significantly (^{A,B}: $p < 0.01$)

Table 4 Total phenolic compound and antioxidant activity in glandless cottonseed, cottonseed kernel and their cold-pressed meal (Mean ± SEM)

Test	Seed		Meal	
	GCS	GCSK	GCSM	GCSKM
Phenolic content (mg GAE/g)	7.87 ± 0.05^{A}	2.18 ± 0.02^{B}	5.86 ± 0.02^{A}	1.91 ± 0.01^{B}
$ABTS^{\cdot+}$ (µmol TE/g)	716.7 ± 0.63^{A}	566.7 ± 0.63^{B}	647.2 ± 0.91^{A}	587.9 ± 0.97^{B}
FRAP (µmol TE/g)	66.07 ± 0.28^{A}	49.79 ± 0.30^{B}	52.23 ± 0.50^{A}	47.27 ± 0.22^{B}
$DPPH^{\cdot}$ (µmol TE/g)	27.39 ± 0.20^{A}	20.40 ± 0.20^{B}	25.66 ± 0.40^{A}	11.29 ± 0.20^{B}
CUPRAC (µmol TE/g)	924.3 ± 4.80^{A}	606.6 ± 9.27^{B}	828.7 ± 7.63^{A}	617.5 ± 13.7^{B}

GCS Glandless cottonseed, *GCSK* Glandless cottonseed kernel, *GCSM* Glandless cottonseed meal, *GCSKM* Glandless cottonseed kernel meal

Means within a row followed by different superscripts differ significantly (^{A,B}: $p < 0.01$)

Table 6 The fatty acid composition of cold-pressed raw cottonseed oil obtained from GCS (g/100 g of total fatty acids; Mean ± SEM)

Fatty acids	Cold-pressed raw cottonseed oil
Lauric acid (C12:0)	0.01 ± 0.003
Myristic acid (C14:0)	0.76 ± 0.012
Pentadecanoic acid (C15:0)	0.03 ± 0.006
Palmitic acid (C16:0)	25.34 ± 0.064
Palmitoleic acid (C16:1)	0.46 ± 0.006
Heptadecanoic acid (C17:0)	0.10 ± 0.006
Heptadecenoic acid (17:1)	0.03 ± 0.003
Stearic acid (C18:0)	2.46 ± 0.023
Oleic acid (C18:1)	14.36 ± 0.043
Linoleic acid (C18:2)	55.55 ± 0.061
α-Linolenic acid (C18:3n3)	0.15 ± 0.003
γ-Linolenic acid (C18:3n6)	0.01 ± 0.000
Arachidic acid (C20:0)	0.31 ± 0.006
Cis-11-eicosenoic acid (C20:1)	0.08 ± 0.003
Eicosadienoic acid (C20:2)	0.04 ± 0.003
Heneicosanoic acid (C21:0)	0.01 ± 0.000
Behenic acid (C22:0)	0.16 ± 0.006
Tricosylic acid (C23:0)	0.02 ± 0.000
Lignoceric acid (C24:0)	0.02 ± 0.000
Nervonic acid (C24:1)	0.10 ± 0.003
ΣSFA	29.22 ± 0.011
ΣUFA	70.78 ± 0.014
ΣMUFA	15.03 ± 0.012
ΣPUFA	55.71 ± 0.017

ΣSFA Total saturated fatty acid, *ΣUFA* Total unsaturated fatty acid, *ΣMUFA* Total mono-unsaturated fatty acid, *ΣPUFA* Total poly-unsaturated fatty acid

Long-term storage of such meals makes the oil bitter, which cause a bad taste and odor due to the excess oil. Therefore, necessary precautions should be taken, because excessive oil in meal can have a negative effect on the feed value. The protein content of cottonseed meal varies depending on the amount of husk and the method of production. The CP content of cottonseed meal ranges between 20 and 45%. The highest CP content in the cottonseed kernel meal on DM was reported 50%, while this ratio can be as low as 20% in the shelled cottonseed meal [28, 29]. NRC [30] reported the CF and EE ratios of cottonseed meal as 13.60% and 0.5%, respectively, while Sahin et al. [20] found the CF and EE ratios as 25% and 8%. Sharma et al. [31], El-Boushy and Raterink [32] and Watkins et al. [33] reported similar CP, CF and ash content in cold-pressed cottonseed meal, except for EE compared with the current cold-pressed GCSKM nutrient contents. The CP, CF and ash content of cold-pressed cottonseed meal reported by Balogun et al. [34] and Nagalakshmi [35] were consistent with those of cold-pressed GCSM. Karataş [36] and Yiğit [37] reported that the DM, OM, CP, EE, CF and ash contents of cottonseed were 91.15, 96.00, 21.04, 20.30, 28.30, 4.00 and 96.00%, respectively. Similarly, Heuzé et al. [28] reported the CP content as 45% in dehulled expeller cottonseed meal, and 37.4% for the samples whose shell was partially removed. Unlike the research findings, the CP content in cottonseed meal samples obtained by mechanical extraction was between 42.96 and 43.05% [38]. The nutrient content is highly dependent on the method of oil extraction, the proportions of fuzzy and linters and the degree of shelling of the seeds [34, 39]. The CF content is an important criterion in determining the quality of cottonseed meal. The high fiber content of cottonseed meal results in relatively low protein digestibility [40] and poor protein quality [41]. Waldroup and Kersey [38] determined the content of CF obtained by mechanical extraction as 9.78–12.27%, which is considerably lower than the current research findings. Other researchers [28, 42] reported that the CF content of cottonseed meal ranged from 10 to 20%; 5 to 25% depending on the level of husk. Heuzé et al. [28] reported that the CF content of cottonseed meal can vary between 5 and 25% depending on whether it is hulled or not. The processing technique applied during the extraction of oil from cottonseed significantly affects the cellulose content of the meal. In addition, the inability to remove the seed shell that appears as cottonseed oil fabrication residues can remain in the meal and increase the CF level. Differences in nutrient composition of cottonseed and meals vary depending on the harvest time (seasonal variety), seed variety, quality, soil type, climate, etc. [43].

4.2 Mineral contents

Minerals present in the body at concentrations ≥ 100 mg/kg body weight are called macro minerals such as sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), phosphorus (P), sulfur (S) and magnesium (Mg). Animals contain small amounts of about 40 minerals at concentrations < 100 mg/kg body weight, which are termed micro minerals or trace minerals [44, 45]. Following 14 micro minerals (iron (Fe), copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), iodine (I), selenium (Se), molybdenum (Mo), chromium (Cr), fluorine (F), vanadium (V), nickel (Ni), boron (B) and bromine (Br)) are considered to have important physiological functions in animals [45]. The micro minerals and the seven macro minerals are classified as nutritionally essential for animals. Their deficiencies cause specific symptoms, such as reduced feed intake, growth restriction, impaired development and even death. However, some of above minerals may be toxic to animals when fed at high levels. Other minerals (e.g., cadmium (Cd), mercury (Hg), lead (Pb),

beryllium (Be), arsenic (As) and aluminum (Al)) are toxic to animals at much lower levels and should be avoided at all times in the diets. The findings related to the mineral concentrations revealed that the values in GCS and GCSM samples were very similar, except for Fe and Pb elements. The highest values in GCS and GCSK were recorded for K, P, Mg, S and Ca elements. The results obtained are in accordance with Calhoun et al. [17] and He et al. [46]. Potassium concentrations in CSM were 1.21 and 1.11%, respectively. The results are consistent with those reported by He et al. [27] who found that the P concentration in cottonseed was between 0.6 and 0.78% and the S concentration between 0.24 and 0.31%. Osti and Pandey [47] reported the P content in cotton seed as 0.75% which was close to the findings of the current study. The P content in cottonseed meal was reported as 0.75% [17]; 0.66% [30]; 1.04% [48]; 1.08% [49] and 0.95% [50] in other studies. The P content for the cottonseed meal was comparatively lower than those of the current results. Similar to the Mg contents of the present study, He et al. [27] reported Mg content of cottonseed between 0.38 and 0.41%. This was similar to the findings of Osti and Pandey [47] who reported 0.16% Ca content in cottonseed. Kaçar and İnal [9] reported that the Ca content in plants varies between 0.2 and 3.0% and the amount of Ca sufficient for most plants is between 0.3 and 1.0%, which is higher than the Ca values of the current study. It could be thought that seed mineral concentrations of plants differ from other vegetative parts of plants. The Ca content in cottonseed meal was reported as 0.12% [49], 0.16% [50], 0.18% [51], 0.22% [41], 0.25% [52] and 0.27% [48]. Kaçar and Katkat [53] claimed that total Na concentrations in plants vary between 0.01 and 10.0%. He et al. [27] stated that the Fe content of cottonseed varied between 32.9 and 42.2 mg/kg. The Fe content of GCS and GCSK was lower than those indicated by others. The Zn concentration of plants varies between 5 and 100 mg/kg, and the toxic effect starts after 400 mg/kg. The Zn levels (0–15 mg/kg) in plants with zinc deficiency are quite low [54]. Copper is taken up by plants in much less quantity (1/10 of Mn) than B, Fe, Mn and Zn. The Cu content of plants varies between 2 and 20 mg/kg [55, 56], which is close to the findings of the current study. The Mn and B content in cottonseed varies from 11.4–12.7 mg/kg to 11.9–14.3 mg/kg which are similar to the previously reported values (11–21 mg/kg and 16.8–20.5 mg/kg) [27]. Two non-nutritive elements, Cd and Pb, contents were extremely low in GCS and GCSM samples. The Al and Pb concentrations in GCSM samples were 4 and 8 times higher than those in the GCS samples. Therefore, the results may be related to Al and Pb contamination from the machine during pressing. He et al. [27] found Cd and Pb values in cottonseed in the range of 0.000–0.003 mg/kg and 0.001–0.017 mg/kg, respectively. The levels of mineral substances in GCS and GCSM samples remained below the maximum tolerable mineral substance levels in farm animals determined by NRC [57].

4.3 Total phenolic compound and antioxidant activity

The cottonseed and cottonseed meal have not been extensively studied as a source of bioactive compounds on phenolic contents and their antioxidant capacity. This is the first study determining the phenolic compounds and antioxidant capacity of GCS and cold-pressed GCSM; therefore, the literature was available to compare with the GCS and their meal. Kumar et al. [58] determined the total phenolic content in *Gossypium herbaceum* seed as 5.86 ± 0.75 mg GAE/g, which is lower compared to the result of the current findings. Zaman et al. [59] determined the total phenolic content in *Gossypium hirsitium* seed as 11 mg GAE/g. On the other hand, Oskoueian et al. [60] found the total phenolic content of cottonseed meal as 1.5 mg GAE/g. Villa et al. [61] determined the total phenolic content in cottonseed as 1.90 g/kg using the Folin–Ciocalteu method. The antioxidant properties can be a valuable feed source for possible nutritional studies on animal health, and production efficiency supports the findings of this study. In this context, corticated cottonseed has been proven to contain more total phenolic components than decorticated cottonseed meal, and its superior antioxidant properties, free radical scavenging effects and ferric ion reducing antioxidant power abilities.

The samples of the cation radical scavenging activity were lower compared to BHA (5178.9 ± 3.51 µmol TE/g) and BHT (3570.2 ± 1.38 µmol TE/g) as a positive control. The GCS and GCSM were superior to the GCSK and GCSKM ($p < 0.01$). Table 4 shows the FRAP of sample extracts resulted in the lowest activity, compared to BHA (5633.8 ± 131.5 µmol TE/g) and BHT (4734.5 ± 95.6 µmol TE/g) standards in the prevention of free radical formation in the environment by reducing the Fe^{3+} ions in the environment of GCS and GCSM samples with and without shells to Fe^{2+}. The total phenolic content and antioxidant activity of cottonseed and cottonseed meal may vary depending on the plant variety, cultivation practices, the growing season, storage conditions, climate and geographical origin and the extraction method used [62]. The results of cottonseed and meal samples on BHA (1643.1 ± 5.49 µmol TE/g) and BHT (738.4 ± 5.29 µmol TE/g) used as standard positive control indicated that $DPPH^{\cdot}$ was very low. Therefore, the $DPPH^{\cdot}$ of glandless cottonseed and meal was very low, and the GCS and GCSM were also superior to the GCSK and GCSKM ($p < 0.01$).

The antioxidant activity was 90.62% at a concentration of 250 µg/mL, based on the 2,2-diphenyl 1-picryl hydrazil free radical scavenging activity in *Gossypium herbaceum* type dry cottonseed, which is called short fiber levant cotton [59]. Oskoueian et al. [60] found the DPPH radical scavenging content of cottonseed meal as 191.1 IC_{50} (µg m/L). Similar results were obtained in the CUPRAC test that synthetic antioxidants BHT (5649.5 ± 56.38 µmol TE/g) and BHA (11185.7 ± 89.51 µmol TE/g) were quite strong efficacy when compared with the current samples.

4.4 Free and total gossypol levels

The free and total gossypol levels tended to increase in GCSK samples since there was no gossypol gland in cottonseed shells. Gossypol concentration is mainly controlled by the genetics of the cotton plant [63]. Some researchers [5, 6] reported that the concentration of gossypol in the seed or meal varies depending on the type of plant, the soil in which it grows, the oil extraction method, linter, seed shell and climate. Price et al. [64] indicated that the gossypol concentration differences in a plant were positively correlated with the precipitation rate and negatively correlated with the temperature. In practice, when 15% cottonseed is added to the laying hen's ration mixes, the ration's gossypol levels will contain approximately 44.1 mg/kg free gossypol and 66 mg/kg total gossypol for GCS; 78.2 mg/kg of free gossypol and 105.9 mg/kg total gossypol for GCSK. The addition 10% in broilers diet will contain approximately 29.4 mg/kg free gossypol and 44 mg/kg total gossypol for GCS; 52.1 mg/kg free gossypol and 70.6 mg/kg total gossypol for GCSK, respectively. Therefore, considering the predicted gossypol levels in the ration, the gossypol level of the poultry is well below the level to be tolerated. The results revealed that the ratio of addition can be increased twice, which will not cause any decrease in growth and egg performance. Davis et al. [65] reported that with the addition of 20% cottonseed meal to the laying hen rations, the free gossypol content (144 mg/kg) in the ration did not affect egg production and egg weight, and suggested that the free gossypol level in the ration should be kept at least 100 mg/kg. Ustaoğlu [25] revealed that the free gossypol levels in all cottonseeds (0.362–0.591%) and the average free gossypol level in expeller cottonseed meal (0.06%) were lower than the gossypol content results of the current study. Karataş [36] and Yiğit [37] reported that cottonseed free and total gossypol levels were 4.23 and 8.47%, and 1.85 and 5.69%, respectively, in heat treated cottonseed.

4.5 Fatty acid content of crude GCS oil

Raw GCS oil contains high levels of linoleic acid (55.55%), palmitic acid (25.34%) and oleic acid (14.36%). The results were similar to those reported by Berberich et al. [66], Nida et al. [67] and Bertrand et al. [68] who indicated that the fatty acid contents in the crude oil of GCS were at the highest level. Total mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) were higher than total saturated fatty acids (SFA) values. The fatty acid composition of the raw GCS oil used in the current study is in agreement with those found by O'Brien [69], Bertrand et al. [68], Konuşkan et al. [70] and Mahesar et al. [71]. The linoleic acid value of cold-pressed raw GCS oil had the highest value (55.55%) compared with those of some experimental results reported by Mahesar et al. [71] and Karahasan [72].

5 Conclusions

The processing technique of dehulling, whether mechanical or extraction, significantly affects the nutrient composition of the meal, especially fat, protein and cellulose. Nazilli glandless cottonseed contains relatively low levels of gossypol, high levels of crude protein and is relatively higher in nutritional properties. The results revealed that the antioxidant activity of phenolic compounds in Nazilli glandless cottonseed and cottonseed meal may contribute to animal health and production efficiency. As a matter of fact, up-to-date information has been created for feed mills and livestock enterprises for the use of glandless cottonseed and its by-product cottonseed meal in compound feed. Further research is needed to improve the feed value of glandless cottonseed and cottonseed meal or to determine the performance and product quality in livestock.

Abbreviations

GCS	Glandless cottonseed
GCSK	Glandless cottonseed kernel
GCSM	Glandless cottonseed meal
GCSKM	Glandless cottonseed kernel meal
DM	Dry matter
OM	Organic matter
CP	Crude protein
EE	Ether extract
NFE	Nitrogen-free extract
CF	Crude fiber
ME	Metabolic energy
GAE	Gallic acid equivalent
TEAC	Trolox equivalent antioxidant capacity
DPPH	1,1-Diphenyl-2-picrylhydrazyl
TE	Trolox equivalent
CUPRAC	Cupric ion reducing antioxidant capacity
ΣSFA	Total saturated fatty acid
ΣUFA	Total unsaturated fatty acid
ΣMUFA	Total mono-unsaturated fatty acid
ΣPUFA	Total poly-unsaturated fatty acid

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Author contributions
AC and AY conceived and designed the study; AY, HE, AC and NG conducted the analyses and contributed to the data collection, drafted the manuscript. All authors read and approved the final manuscript.

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The authors declare that all the data and materials used in this study comply with field standards and are available on demand.

Declarations

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