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Determination of Gossypol Levels of Cottonseed Meal Produced in the Southeastern Anatolia Region

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Highlights:
- Feed analysis
- Cotton seed
- Animal feed

Keywords:
- Cotton seed meal
- Free gossypol
- Total gossypol
- Feed

ABSTRACT:

The levels of free and total gossypol and the raw nutrient content (crude protein, crude oil, dry matter, crude ash, neutral detergent fiber, and acid detergent fiber) in cottonseed meals that are produced in Southeastern Anatolia were determined in the present study. Gossypol and raw nutrient content analyses were carried out by using the cottonseed meal samples obtained from 12 different cottonseed oil factories located in Diyarbakır, Şanlıurfa, Adıyaman, Batman, Mardin, and Gaziantep. As a result of the present study, free gossypol levels in the cottonseed meal samples were 1937, 1878, 1916, 1845, 1442, and 1528 mg/kg, respectively. The free gossypol contents in the cottonseed meal samples obtained from Diyarbakır, Şanlıurfa, Adıyaman, and Batman provinces were statistically significantly higher when compared to samples obtained from Mardin and Gaziantep ($p<0.05$). The crude protein and oil levels of the cottonseed meal samples were analyzed in this study. The results showed that the samples had an average protein content of 26% to 32% and an average crude oil content of 4% to 7%. There was no significant difference among the provinces ($p>0.05$), except for Gaziantep, where the samples had significantly lower oil and significantly higher protein content ($p<0.001$). The analysis also showed that the dry matter content of all samples varied from 88% to 91%, while the crude ash values averaged between 4% and 7%, which are within the standard range.

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INTRODUCTION

Cotton industry by-products (such as cottonseed and cottonseed meal) can be used as alternative feed ingredients in animal feed due to their chemical composition and lower price (Canikli et al., 2023). Cottonseed meal (CSM) is a protein and lipid-rich byproduct of the cottonseed oil industry (Wang et al., 2023), but it contains the sesquiterpenoid gossypol, which has anti-nutritional effects (Aslam et al. 2024). Gossypol is a yellow pigment that has toxic effects on animals, especially at high doses (Gadelha et al., 2014) to many farm animals, especially non-ruminants, because it can be absorbed in the small intestines. Gossypol is present in all parts of the cotton plant, but it is more concentrated in the seeds (Ricci et al., 2015). The seeds have 0.4-1.5% free gossypol, which is the most harmful form, and 2-4% bound gossypol (Pons & Eaves, 1967). In some cases, the gossypol content of cottonseed can reach up to 10% (100 g/kg). Therefore, the use of CSM in animal feed is limited by its gossypol content. However, gossypol can bind to amino acids, mainly lysine, through the ε-amino group and form non-toxic bound gossypol. Bound gossypol is not directly measured, but it is calculated by subtracting free gossypol from total gossypol. Free gossypol is the biologically active and harmful form of gossypol (Hron et al., 1990; Stipanovic et al., 2006; Tegtmeier et al., 2021).

Gossypol is a toxic substance that affects all farm animals in similar ways, but the most noticeable symptoms are breathing problems and reduced appetite. The acute effects of gossypol toxicity include an increase in erythrocyte fragility of heart, lung, liver, and blood cells. Free gossypol was claimed to cause anaemia since it reduces the absorption of iron because it chelates by binding to iron in the small intestine and liver (Braham et al., 1967; Cope, 2018). Post-mortem findings include general edema and obstruction in the lungs and liver, fluid-filled chest and peritoneal cavities, and degeneration in heart muscle fibers. Animals that are given gossypol-containing feeds experience negative effects such as growth retardation due to reduced appetite, reproductive problems, and intestinal and other internal organ abnormalities (Berardi & Goldblatt, 1980; Francis et al., 2001; Robinson et al., 2001; Lv et al., 2024; Wageshwaran et al., 2024). Gossypol toxicity negatively affects reproductive functions in male animals, particularly by reducing sperm mortality, and it also inhibits spermatogenesis, suppresses sperm count, causes toxicity to Sertoli cells, and potentially affects Leydig cells. Moreover, there also are studies reporting that it deteriorates estrous cycles, pregnancy, and early embryo development in monogastric species (Abou-Donia, 1976; Berardi and Goldblatt, 1980; Randel et al., 1992; Dodou, 2005).

Due to its adverse effects, gossypol has been classified as an unwanted substance for animal feeds by the European Union Regulation 574/2011 (European Commission, 2011). Considering the 88% dry matter content, the maximum permissible levels of free gossypol in various feed materials are as follows: 5000 mg/kg for cottonseed, 1200 mg/kg for cottonseed meal, and 20 mg/kg for other feed materials. Specifically, the limit for free gossypol is 500 mg/kg for cattle fattening feeds (excluding calves), 300 mg/kg in sheep and goat feeds (excluding lambs and kids), 100 mg/kg in poultry feeds (excluding laying hens), and 60 mg/kg for pigs (excluding piglets), rabbits, lambs, and kids. The lethal dose (LD50) of gossypol varies across species: 2400-3340 mg/kg in rats, 500-950 mg/kg in mice, 350-600 mg/kg in rabbits, and 550 mg/kg in pigs (EFSA, 2008). Following a request from the European Food Safety Authority (EFSA) for data on gossypol levels in grain or processed cottonseed, information from two member countries indicates that cottonseed byproducts contain gossypol levels ranging from 100 to 8416 mg/kg, while grain cottonseed may have more than 14000 mg/kg of bound and free gossypol (EFSA, 2008).

Accepted consensus acknowledges a data gap concerning gossypol levels (both free and bound) in materials used for animal feed within the European Union (Ricci et al., 2015). Previous studies indicate that gossypol from feeds can transfer to edible tissues, including muscles and offal, in ruminants, poultry, and fish. Rat studies reveal gossypol transfer to milk. While no scientific study directly examines gossypol transfer to eggs or cow milk, it is estimated that cow milk may contain gossypol. However, quantitative information on transfer rates remains limited (EFSA, 2008).

Although adult ruminants exhibit high tolerance to gossypol due to rumen microorganisms binding free gossypol to soluble proteins (Reiser & Fu, 1962; Risco et al., 1992), young ruminants (such as lambs, calves, and kids) and monogastric animals (including pigs, poultry, fish, and rodents) are more sensitive to gossypol-containing feeds (Holmberg et al., 1988; Morgan, 1989; Zhang et al., 2007; EFSA, 2008; Cope, 2018). Consequently, cottonseed or cottonseed meal is either avoided or used at specific limited concentrations in non-ruminant animal feeds (Rathore et al., 2020).

To enhance the utilization of cottonseed meal in monogastric animal diets, it is crucial to assess the free gossypol content. Ongoing research endeavors focus on minimizing free gossypol levels as extensively as possible. Utilizing a screw press (expeller) for cottonseed oil extraction or introducing iron salts to cottonseed meal effectively prevents the absorption of free gossypol by animals, thereby neutralizing its toxic effects (Tanksley, 1990). However, the process of mixing cottonseed meal with iron sulfate at a 1:1 ratio for monogastric animal feeds poses challenges due to factors like varying gossypol concentrations (Rathore et al., 2020) and differences across feed lots. Considering national policies and the imperative to reduce budget deficits while enhancing reliance on domestic protein sources in mixed feeds, it becomes essential to conduct rigorous academic assessments of the gossypol content. This study focuses on cottonseed meal samples obtained from 12 cottonseed oil factories in the Southeastern Anatolia Region, aiming to comprehensively evaluate current gossypol levels.

MATERIALS AND METHODS

Feed Material

In this research, we collected 1 kg of laboratory samples from cottonseed meal produced by a total of 12 factories. We followed specific sampling criteria indicated by name and number during the cottonseed production season (October-November 2018) in the provinces and factories numbers in the Southeastern Anatolia Region (Figure 1). These cottonseed meal samples were transported to the Feeds and Animal Nutrition Laboratory within the Department of Animal Science at Van Yüzüncü Yıl University. The samples underwent grinding, sieving, and were stored at -20 ºC for gossypol analyses by HPLC.

Figure 1. The provinces and the number of factories from which cottonseed meal samples were taken in the Southeastern Anatolia Region

Methods

Determination of crude nutritional contents in cottonseed meal samples

The dry matter (DM), crude ash (CA), and crude protein (CP) content of cottonseed meals were determined according to AOAC (2000), ether extract (EE) content according to AOCS (2005), and acid detergent fiber (ADF) and neutral detergent fiber (NDF) content according to van Soest et al. (1991).

Determination of gossypol in cottonseed meal samples

For total and free gossypol content determination, the cottonseed meal samples underwent extraction in the laboratory. This process involved adapting methods from AOCS (1987a), AOCS (1987b), Hron et al. (1999), and Dowd & Pelitire (2001). During the analysis, approximately 1 g of dried and ground cottonseed meal was used for gossypol extraction. These samples underwent maceration in a 50 ml solution of 70% acetone for 16 hours, followed by filtration through a 0.45-micron Whatman 40 filter paper membrane. The extract was evaporated under vacuum until dry. The residue was then re-suspended in 25 ml of a 1% acetic acid solution. For total gossypol analysis, we prepared a complexing reagent by combining 2 ml of -amino-1-propanol,10 ml of glacial acetic acid, and N,N-dimethylformamide to achieve a total volume of 100 ml. 20 milliliters from this mixture was added to every 1 gram of CSM sample. The samples were incubated at 95-100 ºC for 30 minutes. After cooling to room temperature, 1 milliliter of the sample was combined with 4 milliliters of the mobile phase (85:15 v/v acetonitrile: 10 mM KH_2PO_4, pH adjusted to 3 using H_3PO_4). Finally, 1.5 milliliters of this mixture were transferred to a 2 ml microcentrifuge tube, centrifuged at 12 000 rpm for 2 minutes, and the supernatant was then transferred to a 1.5 ml HPLC vial for analysis using the HPLC device, with an injection volume of 20 µl.

High performance liquid chromatography (HPLC) conditions

The samples were analyzed by following the method introduced by Scheffler & Romano (2008) by using a Shimadzu (Japan) HPLC system, an Inertsil ODS-2 reverse-phase 5 µm column (100 mm x 4.0 i.d), and a DAD detector set to a wavelength of 254 nm and a flow rate of 1 ml/min pump. The calibration curve was established with solutions containing 0, 5, 10, 20, 40, and 80 mg/L standard gossypol in chloroform solution containing 1% acetic acid (Figure 2).

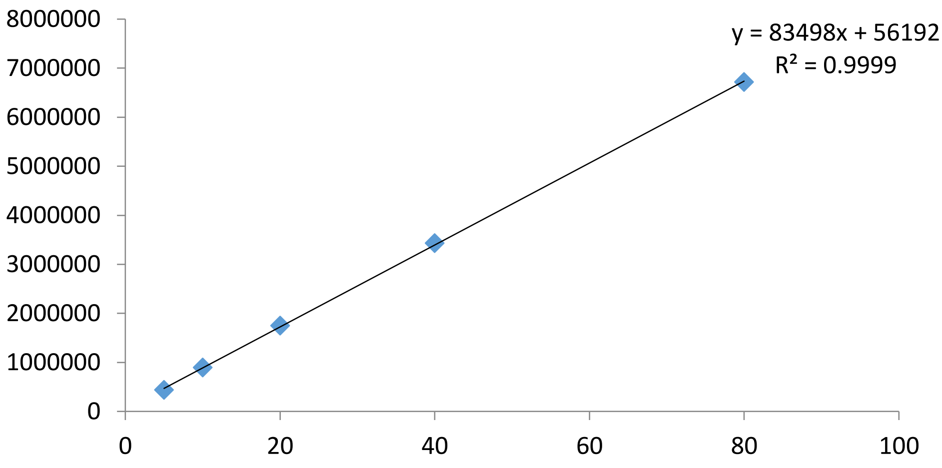

Figure 2. Calibration curve of gossypol standard

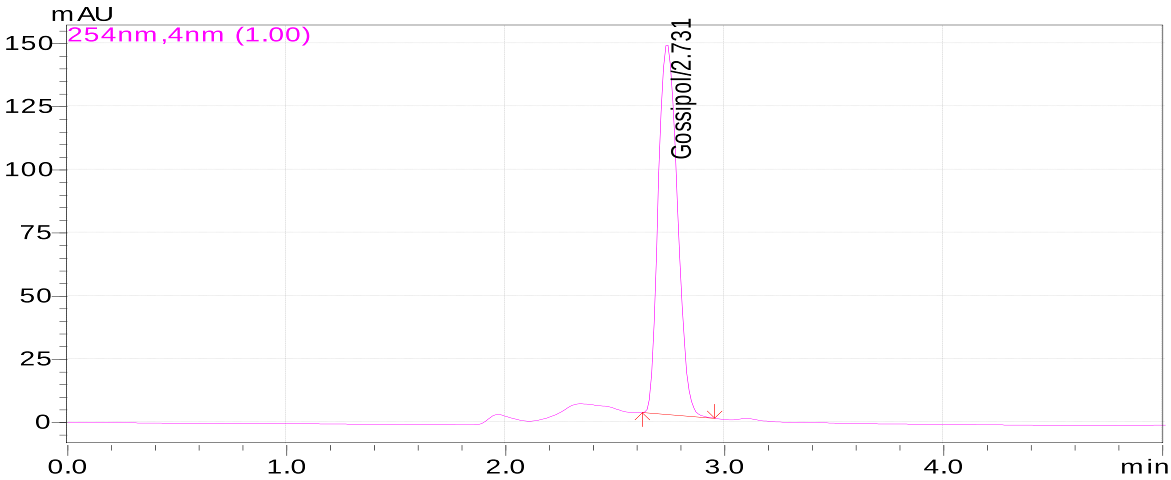

Figure 3. Chromatogram diagram of gossypol standard

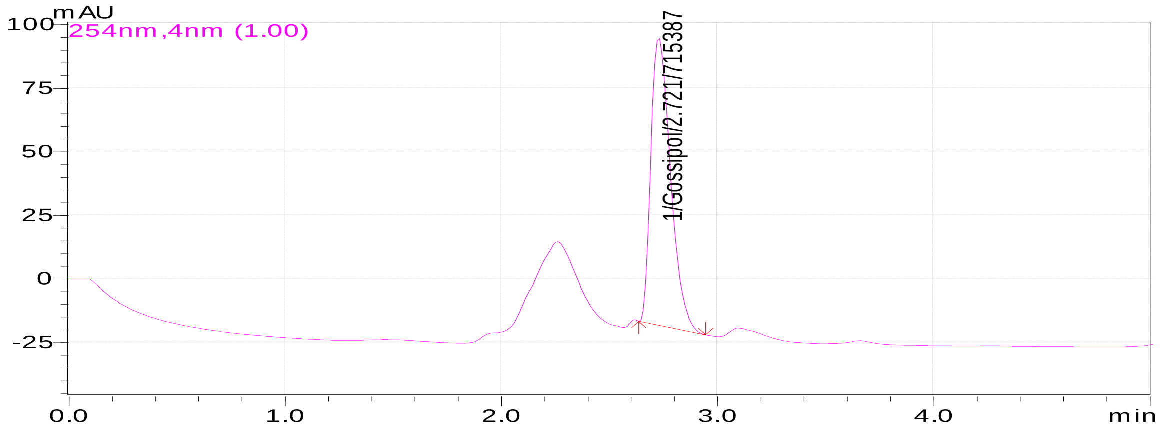

Figure 4. Gossypol chromatogram diagram of CSM sample

Statistical Analyses

Each feed sample was subjected to extraction in six replicates. The mean values and standard error for the gossypol content of the samples were calculated by using SAS (2017) software. One-way analysis of variance (ANOVA) was used in determining the differences between mean values, whereas Duncan's multiple comparison test was conducted to identify differences between factories.

RESULTS AND DISCUSSION

The results of free and total gossypol contents in cottonseed meal samples, obtained from the cottonseed oil factories from each city are presented in Table 1.

Table 1. Free and total gossypol contents of cottonseed meal by provinces

Provinces	**Gossypol contents of cottonseed meal samples mg/kg**					
	Free gossypol			**Total gossypol**		
	Minimum	**Maximum**	**Mean ± SE**	**Minimum**	**Maximum**	**Mean ± SE**
Diyarbakır	1368.29	2313.91	1937.38±69.75[a]	2216.47	5162.79	3217.77±138.02
Şanlıurfa	1647.74	2399.11	1878.85±59.77[a]	2810.65	3891.24	3346.50±95.09
Adıyaman	1730.00	2080.78	1915.77±82.92[a]	2543.86	3337.42	3078.36±184.24
Batman	1675.79	1973.98	1845.31±66.71[a]	3257.92	3937.18	3509.81±149.03
Mardin	1265.26	1609.22	1442.06±70.59[b]	2534.77	3236.36	2849.25±183.25
Gaziantep	1433.39	1653.07	1527.55±46.58[b]	3028.10	3380.35	3234.71±74.21
F		4.15			1.01	
Prp>F		0.0038			0.424	

There are statistically significant differences between the groups in the column and marked with different letters

Table 1 shows that total gossypol content in cottonseed meal does not vary significantly across provinces (p>0.05). However, free gossypol content in samples from Mardin and Gaziantep is significantly lower than in samples from other provinces (p<0.05). Mardin and Gaziantep have comparable free gossypol content levels, as do Diyarbakır, Şanlıurfa, Adıyaman, and Batman, with no significant difference among them (p>0.05).

Examining the factories individually (Table 2), the lowest total gossypol level was found in the CSM samples obtained from Factory 2 in Diyarbakır (2755.77 mg/kg), whereas the highest total gossypol level was found in the samples obtained from Factory 10 in Batman (3509.81 mg/kg) (p>0.05).

Table 2. Free and total gossypol contents of cottonseed meal by oilseed factories

Factory		**Gossypol contents of cottonseed meal samples mg/kg**					
		Free gossypol			**Total gossypol**		
No	**Province**	**Min.**	**Max.**	**Mean ± SE**	**Min.**	**Max.**	**Mean ± SE**
1	Diyarbakır	1652.59	1778.17	1707.77±27.24[de]	2713.06	5162.79	3467.96±578.17
2	Diyarbakır	2012.17	2189.00	2097.87±39.51[ab]	2216.47	3123.03	2755.77±192.74
3	Diyarbakır	1368.29	1593.59	1483.37±59.44[f]	2520.32	3615.49	3011.57±245.49
4	Diyarbakır	2051.36	2242.88	2188.66±45.89[a]	3141.90	3923.88	3465.10±164.63
5	Diyarbakır	1988.61	2313.19	2209.23±74.65[a]	3206.27	3634.38	3388.45±90.26
6	Şanlıurfa	1647.74	1824.54	1738.68±39.93[cd]	2818.65	3891.24	3472.97±229.91
7	Şanlıurfa	1719.67	1937.44	1810.62±45.67[cd]	2878.68	3534.31	3171.27±146.43
8	Şanlıurfa	1900.32	2399.11	2087.27±114.19[ab]	3182.30	3620.88	3395.25±93.83
9	Adıyaman	1730.00	2080.78	1915.77±82.77[bc]	2543.86	3337.42	3078.36±184.24
10	Batman	1675.79	1973.98	1845.31±66.75[cd]	3257.92	3937.18	3509.81±149.03
11	Mardin	1265.26	1609.22	1442.06±70.59[f]	2534.77	3236.36	2849.25±183.25
12	Gaziantep	1625.59	1778.17	1707.77±27.24[de]	3028.10	3380.35	3234.71±74.21
F		17.99			1.26		
Prp>F		0.0001			0.286		

There are statistically significant differences between the groups in the column and marked with different letters

We found that free gossypol contents in samples from Factories 2, 4, 5, and 8 exceeded 2000 mg/kg. This was significantly higher than the free gossypol contents in samples from Factories 1, 3, 6, 7, 10, 11, and 12 (p<0.05). The sample from Factory 11 had the lowest free gossypol content (1442.06 mg/kg). The level of free gossypol limits the use of CSM in compound feeds. Adding CSM with a minimum of 1442 mg/kg free gossypol at a rate of 30% would result in a diet with 432.6 mg/kg of free gossypol. However, adding CSM with a maximum of 2209 mg/kg of free gossypol at the same rate would result in a diet with 662.7 mg/kg of free gossypol. This means that the diet could have 230.1 mg/kg more free gossypol content. Therefore, there is a significant difference in gossypol content among the commercially available cottonseed meals. To achieve the same level of free gossypol content (432.6 mg/kg), we need to add only 19.5% of CSM with a high level of free gossypol. Mbahinzireki et al. (2001) reported that the use of cottonseed meal (CSM) up to a level providing 520 ppm of free gossypol in fish diet does not harm the health of fish. In the present study, CSM obtained from Factory 11 in Mardin, which has the lowest free gossypol content (1442 ppm), can be used at a rate of up to 36% in the diet to achieve the recommended maximum level of 520 ppm of free gossypol. The CSM with the highest free gossypol content (Diyarbakır Factory 5, 2209 ppm) should be used at a rate of 23% in fish diet. In other words, as the free gossypol content in CSM increases, the rate of CSM used in compound feeds should decrease proportionally to avoid any harm to the animals' health.

The free gossypol content level, like the protein level, affects the amount of CSM that can be used in compound feeds. The European Union (EU Regulation 574/2011) (European Commission, 2011) sets the limit for free gossypol in cottonseed meal at 1200 mg/kg and in other feed materials at 20 mg/kg. All the samples from oilseed factories in this study have free gossypol levels above 1200 mg/kg. For cattle fattening feeds (excluding calves), with a free gossypol limit of 500 mg/kg, the highest and lowest rates of CSM from the factories in this study are 34.67% (Mardin) and 22.63% (Diyarbakır Factory 5), respectively. For sheep and goat feeds, these rates are even lower: 20.80% (Mardin) and 13.58% (Diyarbakır Factory 5). The data on gossypol levels in all seed grains and cottonseed by-products show a range of 100 to 8416 mg/kg (EFSA, 2008). The CSMs used in this study have total gossypol levels below 8416 ppm and even below 5000 ppm. Tuncer & Yalçın (1986) also reported that the production method of cottonseed meal influences the gossypol levels. Expeller-pressed cottonseed meal has lower gossypol levels than extracted cottonseed meal. The free gossypol levels they found were 300, 400, and 2400 mg/kg for expeller, press, and extracted cottonseed meals, respectively. In this study, we observed that two factories (Diyarbakır Factory 5 and Gaziantep Factory 12) use the extraction method, while the others use the expeller method to extract oil. The CSM sample from Diyarbakır Factory 5 has a free gossypol content of 2209.23±74.65 mg/kg, which is close to the 2400 mg/kg value. The CSM sample from Gaziantep Factory 12 has a lower free gossypol content of 1527.55±46.57 mg/kg. The samples from the other 10 factories in this study had free gossypol contents (around 1400-2000 mg/kg) that were much higher (4-5 times) than the free gossypol contents that Tuncer & Yalçın (1986) found for expeller-pressed cottonseed meal (400 mg/kg). This shows that the findings of this study did not match those of the previous study.

This study measured the CP (%) and EE (%) levels in cottonseed meal samples. The average CP content for all cities were between 26-32%, while the average EE content (except for Gaziantep) was 4-7% (Table 3). The crude protein contents of cottonseed meal samples from Diyarbakır, Şanlıurfa, Adıyaman, Batman, and Mardin were not significantly different ($p>0.05$). However, the cottonseed meal sample from Gaziantep had significantly lower oil and higher protein content than the samples from other provinces ($p<0.001$). Umur et al. (2019) used the HPLC method to measure the gossypol contents of cottonseed meals with different protein contents. They found that cottonseed meals produced in 2013 and 2014 with protein contents of 10.79-36.40% had (+) and (-) gossypol isomers and total gossypol levels of 30.26-1134.00 mg/kg, 37.21-933.32 mg/kg, and 72.37-2285.38 mg/kg, respectively. The protein contents in this study were like those in the previous study (26-32%), The crude protein level of the cottonseed meals in this study was higher than the average crude protein level (22.1% CP) reported by Yeşil (2010) for 10 cottonseed meal samples.

Bayındır (2015) measured the crude protein and oil levels of 40 cottonseed meal samples in Konya-Türkiye. The crude protein levels ranged from 12.4% to 34.5% (average 28.7%, CP), and the ether extract levels ranged from 0.47% to 7.25% (average 3.2%, EE). The average crude protein level in this study for all provinces was 28.04%, which is very close to the Konya CSM results.

Table 3. Crude protein and ether extract levels of cottonseed meal samples by provinces

Provinces	CP (%)			EE (%)		
	Min	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**
Diyarbakır	24.76	32.79	28.59±0.47[b]	0.97	8.74	4.96±0.53[b]
Şanlıurfa	24.95	30.08	26.94±0.52[b]	5.43	10.57	7.60±0.46[a]
Adıyaman	26.46	27.24	26.83±0.20[b]	5.40	6.89	6.13±0.38[ab]
Batman	26.48	28.01	27.15±0.32[b]	1.91	6.05	4.25±0.90[b]
Mardin	24.89	27.98	26.36±0.69[b]	4.00	6.05	5.13±0.46[b]
Gaziantep	30.53	33.81	32.42±0.71[a]	1.38	1.79	1.54±0.092[c]
F	7.35			7.40		
Prp>F	0.0001			0.0001		

There are statistically significant differences between the groups in the column and marked with different letters

The cottonseed meal sample from Factory 12 had a much higher % protein level than the samples from other provinces ($p<0.05$). The other samples had similar protein levels (Table 4). The samples from Factories 5 and 12, which used the extraction method, had very low oil levels (less than 2%, $p<0.001$). The sample from Factory 8 had oil significantly more than the samples from other Factories ($p<0.05$). The oil levels of the samples from Factories 1, 2, 3, 4, 6, 7, 9, and 11 were not significantly different ($p>0.05$).

The EE contents of cottonseed meal samples extracted with solvent (below 2%) matched the standard values. However, the crude protein contents of these samples (32.42% and 30.20%) were still below the average 41% standard CP value for cottonseed meal with extracted oil (NRC, 1994). He et al. (2015) reported that the crude protein content of cottonseed meals ranged from 30% to 50%, which agrees with the values in this study for solvent-extracted samples. Nagalakshmi et al. (2007) also found that the crude protein values of cottonseed meals varied from 22% to 56%, which is in line with the findings of this study.

Table 4. Crude protein (%) and ether extract (%) contents of CSM samples by factories

Factory		CP (%)			Oil (EE) (%)		
No	Province	Min	Max	Mean ± SE	Min	Max	Mean ± SE
1	Diyarbakır	26.32	30.76	28.98±1.03[bc]	5.53	5.88	5.76±0.08[bc]
2	Diyarbakır	24.76	28.67	27.00±0.87[cd]	5.61	5.97	5.84±0.07[bc]
3	Diyarbakır	29.79	30.55	30.12±0.15[b]	4.62	8.35	6.55±0.76[b]
4	Diyarbakır	25.02	27.68	26.64±0.58[d]	2.97	8.74	5.44±1.45[bc]
5	Diyarbakır	28.28	32.79	30.20±1.03[b]	0.97	1.72	1.24±017[d]
6	Şanlıurfa	24.95	26.42	25.88±0.32[d]	6.37	7.48	6.88±0.25 [b]
7	Şanlıurfa	26.73	30.08	29.04±0.77[bc]	5.43	8.84	6.68±0.75[b]
8	Şanlıurfa	24.95	26.42	25.89±0.32[d]	8.30	10.57	9.26±0.56[a]
9	Adıyaman	26.46	27.24	26.83±0.20[d]	5.40	6.89	6.13±0.38[bc]
10	Batman	26.48	28.01	27.15±0.31[cd]	1.91	6.05	4.25±0.90[c]
11	Mardin	24.89	27.98	26.36±0.68[d]	4.00	6.05	5.13±0.46[bc]
12	Gaziantep	30.53	33.81	32.42±0.71[a]	1.38	1.79	1.54±0.092[d]
F			9.97			12.8	
Prp>F			0.0001			0.0001	

There are statistically significant differences between the groups in the column and marked with different letters

The average dry matter (DM) and crude ash (CA) contents of cottonseed meal by provinces and factories are given in Tables 5 and 6.

Table 5. Dry matter (%) and crude ash (%) contents of CSM samples by provinces

Provinces	DM and CA contents of CSM samples					
	DM (%)			CA (%)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Diyarbakır	88.14	92.28	90.59±0.60[ab]	4.42	6.12	5.18±0.09[bc]
Şanlıurfa	88.30	93.92	91.57±0.41[a]	4.78	6.48	5.77±0.19[b]
Adıyaman	88.91	91.86	90.85±0.68b[a]	5.52	8.42	7.14±0.72[a]
Batman	91.14	91.37	91.23±0.05[a]	4.65	5.08	4.84±0.09[c]
Mardin	85.26	90.91	88.71±1.36[c]	4.73	5.09	4.93±0.09[c]
Gaziantep	88.31	91.11	89.05±0.69[bc]	4.80	5.18	5.05±0.09[bc]
F		3.83			9.89	
Prp>F		0.0006			0.0001	

There are statistically significant differences between the groups in the column and marked with different letters

Table 6. Dry matter (%) and crude ash (%) contents of CSM samples by factories

actory		DM and CA contents of CSM samples					
		DM (%)			CA (%)		
No	**Province**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**
1	Diyarbakır	89.81	91.75	90.83±0.43[ab]	4.42	5.00	4.63±0.13[d]
2	Diyarbakır	90.59	90.96	90.84±0.09[ab]	5.13	5.24	5.20±0.03[cd]
3	Diyarbakır	92.11	92.27	92.20±0.04[a]	5.04	5.32	5.14±0.06[cd]
4	Diyarbakır	88.14	89.41	88.98±0.29[bc]	4.92	6.12	5.52±0.31[bcd]
5	Diyarbakır	90.02	90.17	90.10±0.04[abc]	5.22	5.67	5.40±0.09[cd]
6	Şanlıurfa	88.30	93.92	92.02±1.26[a]	6.03	6.48	6.30±0.09[b]
7	Şanlıurfa	90.72	92.10	91.15±0.32[a]	4.78	6.24	5.22±0.34[cd]
8	Şanlıurfa	91.27	91.66	91.53±0.08[a]	5.21	6.39	5.80±0.29[bc]
9	Adıyaman	88.91	91.86	90.85±0.68[ab]	5.52	8.42	7.14±0.72[a]
10	Batman	91.14	91.37	91.23±0.05[a]	4.65	5.08	4.84±0.08[d]
11	Mardin	85.26	90.91	88.71±1.36[c]	4.73	5.09	4.93±0.09[cd]
12	Gaziantep	88.31	91.11	89.05±0.69[bc]	4.80	5.18	5.05±0.08[cd]
F			3.47			6.68	
Prp>F			0.002			0.0001	

There are statistically significant differences between the groups in the column and marked with different letters

Tables 5 and 6 show that Şanlıurfa had the highest average DM by province (91.57%), while Mardin had the lowest (88.71%) ($p<0.001$). The samples from Factory 3 in Diyarbakır and Factories 1, 2, 5, 6, 7, 8, 9, and 10 had similar DM percentages ($p>0.05$). The highest crude ash % by province was in Adıyaman (Factory 9, 7.14%), and the lowest was in Batman (Factory 10, 5.08%) ($p<0.001$). This study found that the samples had DM contents of 88-91% and crude ash values of 4-7%, which matched the standard DM and crude ash values. Canikli et al. (2023) determined DM in cotton seed meal obtained by cold pressing as 94.99% and crude ash as 4.65%.

Tables 7 and 8 show the NDF and ADF values of cottonseed meal samples by province and factories. The samples from Diyarbakır had the lowest NDF average (47.11%), while the samples from Şanlıurfa had the highest (59.29%). However, this difference was not statistically significant. Bayındır (2015) measured the NDF values of cottonseed meal samples and found them to range from 32.52% to 51.24%, with an average of 41.53%. The NDF values in this study (47.11-59.29%) were higher than those in the previous study.

The ADF values of the CSM samples varied across the provinces, from a low of 12.84% to a high of 45.62%. The CSM sample from Gaziantep had a significantly lower average ADF value (27.01%) than the CSM samples from Mardin, Batman, Adıyaman, and Şanlıurfa provinces ($p<0.05$). Bayındır (2015) found that the ADF value of CSM samples ranged from 24.44% to 31.11%, with a mean of 27.11%. The current study's values are slightly different from the previous study's values. The ADF values of the CSMs from Factories 1, 8, and 9 did not differ significantly from each other ($p>0.05$), but the CSMs from Factories 2, 3, 5, 7, and 12 had significantly different ADF values among them ($p<0.05$).

Table 7. Average NDF (%) and ADF (%) values of CSM samples by province

Province	NDF and ADF contents of CSM samples					
	NDF (%)			ADF (%)		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Diyarbakır	41.06	53.38	47.11±1.26	12.84	45.50	33.42±1.42[ab]
Şanlıurfa	54.14	62.76	59.29±1.47	22.90	45.62	37.31±1.61[a]
Adıyaman	43.62	46.26	45.23±0.57	36.59	39.39	38.51±0.65[a]
Batman	47.06	50.61	47.89±1.12	34.45	39.44	36.72±1.11[a]
Mardin	51.18	56.99	50.26±1.29	33.27	37.34	35.28±0.86 [a]
Gaziantep	47.82	55.72	51.38±2.09	19.96	31.58	27.01±4.65[b]
F		2.71			2.91	
Prp>F		0.06			0.007	

There are statistically significant differences between the groups in the column and marked with different letters

Table 8. Average NDF (%) and ADF (%) levels of CSM samples by factories

Factory		NDF and ADF contents of CSM samples					
		NDF (%)			ADF (%)		
No	Province	Min	Max	Mean ± SD	Min	Max	Mean ± SD
1	Diyarbakır	41.06	49.95	44.62±3.09	12.84	45.50	37.99±1.12[a]
2	Diyarbakır	44.64	52.27	48.73±1.60	12.81	33.75	27.10±4.82[b]
3	Diyarbakır	42.74	52.56	47.72±2.03	27.53	37.07	32.49±2.16[b]
4	Diyarbakır	50.25	53.38	52.19±2.61	32.94	45.50	36.60±2.98[ab]
5	Diyarbakır	50.17	54.64	52.29±0.99	28.45	35.23	32.91±1.57[b]
6	Şanlıurfa	44.14	52.76	49.33±1.83	35.40	40.53	37.90±1.07[ab]
7	Şanlıurfa	46.74	52.48	49.85±1.23	22.90	38.75	32.64±3.42[b]
8	Şanlıurfa	47.36	50.35	49.33±0.62	38.47	45.62	41.36±1.71[a]
9	Adıyaman	43.62	46.26	45.23±0.57	36.59	39.39	38.51±0.65[a]
10	Batman	47.06	50.61	47.89±1.12	34.45	39.44	36.72±1.1[ab]
11	Mardin	51.18	56.99	50.26±1.29	33.27	37.34	35.28±0.86[ab]
12	Gaziantep	47.82	55.72	51.38±2.09	19.96	31.58	27.01±2.65[b]
F			4.98			3.60	
Prp>F			0.17			0.00017	

There are statistically significant differences between the groups in the column and marked with different letters

CONCLUSION

The study examined the total and free gossypol levels in cottonseed meal samples from 5 provinces and 12 factories in the Southeastern Anatolia Region. It found that the gossypol levels varied by province. This means that some cottonseed meals from one province may be safe for monogastric animals, while others from another province may be toxic. The European Union (EU Regulation 574/2011) (European Commission, 2011) sets a limit of 1200 mg/kg for the free gossypol content of cottonseed meal (CSM). However, the CSM samples from this study had higher free gossypol levels than the limit. According to EFSA (2008), the gossypol content of all cottonseed by-products ranges from 100 to 8416 mg/kg. The current study's results show that the CSM samples had a relatively low gossypol content, with less than 5000 mg/kg of total gossypol. The free gossypol content of CSM affects how much CSM can be used in feed. Since large poultry companies use CSM as an alternative to soybean meal, regular gossypol analysis in feed will help reduce soybean imports and save foreign currency. The study concluded that all CSM samples met the expected standards for raw nutrient components. It also found that the crude protein percentage in cottonseed meal.

The raw nutrient component of all cottonseed meal samples were in arrange level of standard values and met the expected criteria. The crude protein percentage in cottonseed meal was between 26% and 32%, and the amount of cottonseed meal that could be added to feed differed by province.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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