Evaluation of Correlations Between Nutrients, Fatty Acids, Heavy Metals, and Deoxynivalenol in Corn (*Zea mays* L.)

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Primary Audience:

SUMMARY

This study was conducted to develop linear regression equations to estimate nutrients, fatty acids, heavy metals, color characteristics (L,* a,* b*), and deoxynivalenol (DON) level of corn grains ($n = 54$) collected from different feed mills in Turkey. Procc corr and reg procedures were used to analyze the data. Among the nutrients, asit detergent lignin, Ca, and P had the highest variability. The heavy metal concentrations (Cd, Cr, Cu, Ni, Pb, and Zn) were lower than certain limits posted by regulation agencies for animals. Occurrence of DON was 53%, with the highest level of 0.725 ppm. Total of 32 highly significant ($P < 0.01$) correlations among nutrients, fatty acids, heavy metals, and color characteristics were determined in the current study. Correlations for estimating the range of parameters measured in corn from color characteristics lacked the practical importance. The correlations between C18:2 C18:1, ADF−NDF, Zn−Ca, Pb−Cu, and Pb−Cd produced the highest R (0.64 to 0.87) and R^2 (0.41 to 0.74) values that would be of practical importance. The research showed that there are significant correlations among different components in corn grains that could provide necessary information to both plant breeders and feed manufacturers in the field.

Key words: corn, correlation, deoxynivalenol, fatty acid, heavy metal, nutrient

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DESCRIPTION OF PROBLEM

Corn (*Zea mays* L.) is a cereal plant that was first cultivated by indigenous Americans in North America [1]. Nutrient composition of

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corn reported to be influenced by the type of seed, soil composition, and especially weather conditions that can change from year to year [2]. Dry matter content of corn varies between 25% and 30% approximately at harvest that is very high for proper management of storage and, therefore, for animal nutrition [3]. The use of air drying to increase the DM content of corn has been the common industry practice to overcome the negative effects of this high moisture for decades. However, large variations in the temperatures (from 50°C to 130°C) used among facilities when drying corn may also affect its nutrient availability to animals, in addition to a wide variety of factors effecting its nutrient composition [4]. Hence, previous studies reported high variability on CP (6.4%−13.4%, DM-basis), EE (2.0%−7.7%, DM-basis), NDF (7.6−22.3, DM-basis), Ca (<0.001%−0.03% as-is), and P (0.18%−0.26%, as-is) content of corn [5–9]. When formulating animal diets, precise nutrient content knowledge of ingredients is necessary in order to ensure that optimum animal performance is achieved as a result of utilizing more nutrients. Therefore, data on the nutrient content of corn need to be updated frequently to formulate diets more precisely.

Heavy metals can come from different varieties of sources including industrial practices, waste water usage, and fertilizer application in agriculture and pose a great threat to the health of both humans and animals [10]. High concentrations of heavy metals have been found in both water sources and plants because of extensive usage of pesticides containing heavy metals [11]. Moreover, the extensive use of both synthetic and natural sources of fertilizers in agriculture has resulted in excessive accumulations of heavy metals such as Cd, Cu, Pb, and Zn in soil that increases the uptake of these heavy metals by plants [12]. Therefore, frequent and detailed heavy metal content monitoring of corn and other cereals should be implemented to reduce heavy metal intake of both animals and humans.

Deoxynivalenol (DON), also called as vomitoxin, is a mycotoxin belongs to tricotesene family and known to be present in most cereals consumed by both humans and animals [13]. Barley, wheat, and corn have been reported to be the most frequent sources of DON among cereals [14]. A wide range of detrimental effects of DON have been reported in the literature including decrease in live performance and the number of offspring produced, immunotoxicity, DNA damage in lymphocytes in different animal species depending on specific host resistance [15-18]. In a recent study, Kos et al. [19] monitored the occurrence of DON in corn in Serbia, with a total of 1,800 samples collected during the years of 2013−2015, and the percentage of positive samples reported were 2.5% for 2013; 96% for 2014; and 15.5% for 2015, respectively. Kos et al. [19] reported that the extremely rainy weather during 2014 was the reason for the high DON occurrence in corn that year. Therefore, close monitoring of DON levels in corn and other cereals destined for both human and animal consumption is required to minimize problems associated with its occurrence.

Despite considerable data reporting the nutrient content of corn, to the best of our knowledge, no data are available regarding evaluation of correlations between nutrients, color characteristics, heavy metal content, and DON level. Therefore, the aim of the current study was to screen all possible correlations among nutrients, heavy metals, color measurements, and DON concentrations to develop prediction equations that could be used in practice for breeding or feed formulation purposes.

MATERIALS AND METHODS

Corn Samples

The corn samples ($n = 54$) used in the current study were gathered from different feed mills located in Turkey between May 2015 and March 2016. Special attention was paid to collect samples with a wide variety of color characteristics ranging from light yellow to red in visual appearance. Approximately 1 kg sample were taken from each source and sealed in plastic bags and immediately stored at −18°C until analysis.

Color Measurement

Corn samples were ground to pass through a 0.5 mm screen [20] and were placed in a petri dish (90-mm diameter) to a depth of approximately 1.5 cm before color measurements taken. Measurements were taken from 3 different locations on the outer surface of each corn sample with a Minolta chromameter [21] using the standard CIELAB color system ($L^* =$ lightness, $a^* =$ redness, and $b^* =$ yellowness). The chromameter settings were as follows: 8 mm aperture size, 2° angle of observation, and illuminant D65 as the light source. A standard ceramic tile was used to calibrate the chromameter.

Near-Infrared Reflectance Spectroscopy

Near-infrared reflectance spectroscopy [22] was used to estimate DM, CP, EE, crude ash, ADF, NDF, and CF content of corn samples. The data set used for the calibration of NIRSystem contained 150 corn samples that had been previously analyzed by a private feed mill. The calibration data set for corn used in NIRSystem did not include the validation samples used in the current study. Wavelengths from 1,100 to 2,500 nm were used for the prediction of a variable with the NIRSystem. Reflectance was taken at 2-nm intervals. The data of spectra were transformed to log (1/R) using a commercial software (WINISI II version 1.02, Infrasoft International, Port Matilda, PA, USA).

Proximate Composition

Samples of corn were analyzed [23] to determine DM by the oven drying method (930.15), cruse ash in a muffle furnace (942.05), CP by the Kjeldahl method (984.13), EE by the Soxhlet method (920.39), ADF, NDF, and acid detergent lignin (ADL) by a filter bag technique (978.10; 973.18; 978.18D). Starch content was measured with a polarimeter by determining optical activity of a resulting solution obtained from the extraction of corn samples [24].

Fatty Acid Analysis

After the extraction of EE content gravimetrically by the Soxhlet method [23], obtained fat samples per corn sample was frozen at −18°C until they were analyzed for fatty acid composition. The fat samples were mixed with 4 mL anhydrous diethyl ether (with 100 ppm BHT) and then transferred to Teflon-capped tubes followed by evaporation of the solvent. Then, 5 mL of 2 N NaOH-methanol mixture was added and this mixture was placed in a water bath at 75°C for 20 min. Then, 2 mL of borontrifluoride-methanol (20%, w/w) was added and incubated in a water bath at 75°C for another 20 min. Fatty acid methyl esters (**FAME**) were extracted with 1.5 mL of hexane and 2 mL of saturated sodium chloride. Following centrifugation at 4,000 × g for 5 min, upper phase was collected and transferred to a clean glass vial before gas chromatography. A gas chromatograph [25] with a flame ionization detector was used to analyze the FAME. A 30 m × 0.25 mm i.d. colon [26] was used for the FAME analysis. The initial temperatures of injector, detector, and oven were 240°C, 240°C, and 185°C, respectively. The temperature of the oven increased to 240°C with 5°C increases per min and held at this temperature for 1 min. One μL sample was injected into the gas chromatograph with a 1:100 split ratio. Each FAME was identified by comparing their retention times with the FAME standards [27].

Heavy Metal Analysis

Approximately 0.25 g corn sample was placed in a Teflon digestion vessel and samples digested in HNO_3 (65%) and NH_4F (10%) for 30 min at 170°C in a microwave oven [28]. After cooling the samples to approximately 40°C, they were diluted to 25 mL with distilled water and transferred to test tubes before inductively coupled plasma (ICP) spectroscopy readings. Then, the mineral (Ca, Cd, Cr, Cu, Ni, Pb, and Zn) concentrations were determined by an ICP/Mass spectrometry [29]. Phosphorus content from the same digested and diluted samples was determined through the formation of a phospho-molybdenum complex using acid molybdate and Fiske−Subbarow reducer solutions followed by reading absorbance at 630 nm [23] (method 946.06) in a plate reader [30].

Deoxynivalenol Analysis

Deoxynivalenol analysis was performed as described by Klötzel et al. [31] at Tubitak MAM laboratories [32]. Briefly, finely ground corn samples (25 g) were extracted with 200 mL distilled water using an Ultra Turrax homogenizer

Table 1. Descriptive statistics for the nutrient composition (%) and color characteristics (l*, a*, b*)[1] of corn samples (as-is)[2].

Item	Method	Minimum	Maximum	Mean	SD[3]	CV[4]
DM	Wet[5]	85.6	89.8	88.0	1.10	1.25
	NIRS[6]	84.1	88.6	86.1	1.08	1.26
CP	Wet	6.6	9.7	7.5	0.60	7.90
	NIRS	6.6	8.2	7.2	0.33	4.55
EE	Wet	2.2	4.1	3.2	0.46	14.55
	NIRS	2.1	3.5	2.8	0.30	10.70
Ash	Wet	0.8	1.3	1.1	0.11	9.93
	NIRS	1.1	1.2	1.2	0.02	2.13
ADF	Wet	1.9	3.1	2.6	0.24	9.13
	NIRS	2.0	2.1	2.1	0.03	1.55
NDF	Wet	7.9	11.6	9.7	0.88	9.1
	NIRS	7.8	8.9	8.3	0.27	3.25
ADL	Wet	0.4	1.9	0.9	0.3	33.73
CF	NIRS	1.4	1.8	1.7	0.07	3.92
Starch	Wet	65.50	73.63	68.28	1.32	1.93
Ca	Wet	0.06	0.24	0.08	0.03	33.94
P	Wet	0.17	0.33	0.23	0.03	15.00
L*		84.4	89.1	86.8	1.01	1.17
a*		0.3	2.6	1.2	0.49	39.35
b*		23.8	35.7	28.7	2.45	8.52

[1] L* = lightness, a* = redness, b* = yellowness.

[2] The values based on analysis of duplicate and triplicate samples for nutrients and color characteristics, respectively (n = 54).

[3] SD = standard deviation.

[4] CV = coefficient of variation.

[5] Wet = refers to chemical analysis.

[6] NIRS = near infrared reflectance spectroscopy.

[33] at 11,000 rpm for 3 min. The extract was then centrifuged at 10,000 rpm for 5 min and passed through a filter paper [34]. Two mL of the extract was then applied onto an immunoaffinity column [35]. The column then was washed with 5 mL of distilled water at one drop/s and dried by passing air through it with a sterile syringe. Deoxynivalenol was eluted from the columns with methanol 4 times (2 × 250 μL and 2 × 500 μL) with 3 min holding time after each elution. The eluate was then evaporated under a nitrogen gas stream at 40°C. Then, 100 μL of the final eluate was used to determine DON concentrations by HPLC [36] that was equipped with a UV-vis detector [37].

Statistical Analysis

Data were subjected to simple linear regression using PROC REG and Pearson correlation (R) analysis was conducted by using PROC CORR statements in SAS [38].

RESULTS AND DISCUSSION

Nutrient Composition of Corn Grains

Nutrient composition of corn generally depends on seed variety, soil it was planted, and the weather conditions related to the year it was harvested [2]. Therefore, continuous monitoring of the nutrient content of corn is necessary to ensure consistent product quality, regardless of the purpose of its usage in the industry. The average DM content of 88% was very satisfactory to safely store and handle corn grains in the current study (Table 1). The CP content of the corn samples ranged from 6.6% to 9.7%, with an average of 7.5% (as-is). The average CP content of the corn samples was about 12% and 9% lower (7.5% vs. 8.5% and 8.2%) than the CP content reported by NRC [7, 39]. However, the CP levels (minimum and maximum) reported in the current study were within the reported ranges (5.7%–11%, as-is) by other studies [40–43]. The EE content was about

Table 2. Descriptive statistics for fatty acid composition (% of total fatty acids) and heavy metal content (mg/kg) of corn samples (as-is)[1].

Item	Minimum	Maximum	Mean	SD[2]	CV[3]
Palmitic acid (C16:0)	8.08	13.13	10.90	1.11	10.21
Stearic acid (C18:0)	0.80	2.83	1.49	0.43	28.58
Oleic acid (C18:1)	23.66	34.41	28.68	2.25	7.86
Linoleic acid (C18:2 n-6)	52.35	65.76	57.75	2.57	4.45
Linolenic acid (C18:3 n-3)	0.70	1.48	1.19	0.18	14.85
Cd	0.02	0.08	0.03	0.02	50.49
Cr	0.34	1.59	0.76	0.25	33.23
Cu	1.58	5.80	2.52	0.80	31.69
Ni	1.31	11.34	2.56	1.39	54.07
Pb	0.35	1.05	0.50	0.16	31.33
Zn	18.16	63.82	27.48	8.66	31.53

[1] The values reported for fatty acids (n = 54) and heavy metals (n = 50) are mean of two analyses per sample.

[2] SD = standard deviation.

[3] CV = coefficient of variation = [(SD/mean) × 100].

15%–9% lower (3.2% vs. 3.8% and 3.5%) than the EE levels reported by NRC [7, 39]. Crude ash values were found to range between 0.8% and 1.3% with an average of 1.1% that is very close to the crude ash value of 1.3% reported by the NRC [7]. The average ADF and NDF values of 2.6% and 9.7% were slightly lower than those reported by NRC [44], which showed an average of 3.3% for ADF and 10.8% for NDF, respectively. Starch is mainly located within the cells of endosperm of the seed, and corn is one of the most abundant and valuable starch sources for both humans and animals throughout the world [45]. The average starch content of the 54 corn samples was 68% and ranged between 65% and 74% on an as-is basis. Moreover, the starch content of corn grains showed relatively low CV (1.93) when compared to other nutrients in the current study. The average starch content (78%, DM-basis) reported in the current study was around 7%–15% higher than the reported values between 67% and 73% (DM-basis) by others [8, 42, 46]. Corn Ca values (0.06%–0.24%, as-is) in the present study were considerably higher than values (<0.001%–0.03%, as-is) in the literature [6, 7, 39, 41]. Our results indicated that there is extreme variation in the Ca content of corn grains, which is in line with the other reports. However, the substantial variation in the Ca content of corn grains is expected to be negligible because of its very low total levels for human and animal diets. The quite high variation for Ca content in corn grains between studies, at least, could be due to different protocols used between laboratories. Phosphorus is a significant contributor to feed cost after energy and protein and inorganic sources such as monocalcium phosphate and dicalcium phosphate have been the biggest contributor to the overall total P content of diets. Therefore, optimizing the inclusion of inorganic P sources in the diet of animals by having a precise data on P content of ingredients in diets is crucial. The average P content of 0.23% (as-is) in the current study showed good agreement with Lee et al. [47] and NRC [7], who reported means of 0.22% and 0.26% for total P content of corn on an as-is basis, respectively. The obtained CV value of 15 for the P content of corn grains in the current study also agrees with the CV values of 14.5 and 19.2 reported by Lee et al. [47] and NRC [7], respectively.

Fatty Acid Composition

C18:2 was the predominant (57.7%, Table 2) fatty acid in the corn grains followed by C18:1 (28.7%) and C16:0 (10.9%), and its level is in good agreement with previous reports [48, 49]. However, the upper (1.48%) and lower (0.70%) limits obtained for C18:3 content in the current study were markedly lower than the upper (2.1%) and lower (1.20%) limits reported by Goffman and Böhme [48].

Heavy Metal Content

Cadmium is an extremely abundant toxic metal and the crops destined for consumption by animals and humans could be easily contaminated by this heavy metal [50]. Different industrial applications and increasing agricultural herbicide, pesticide, and fertilizer usage have been reported as some of the causative sources for the accumulation of Cd in the environment and plants [51, 52]. Plants grown on soil naturally or artificially contaminated with Cd have been reported to be the second leading source of this heavy metal for humans, after cigarette smoking [53]. The highest Cd level (0.08 ppm) determined in the current study (Table 2) was found to be below the permitted concentration limits of 0.10 and 0.20 ppm for humans proposed both by the codex alimentarious commission [54] and the European Union (EU) [55], respectively. Moreover, all the analyzed corn samples had lower Cd concentrations than the 10 ppm maximum tolerable level set for poultry [39]. Therefore, the current data indicated that there was no health risk related to Cd exposure by corn consumption in both humans and animals.

Chromium is one of the heavy metals whose concentration in the environment is still high. Its common usage in industries such as stainless steel, non-iron alloy, leather, and mining throughout the world is the reason for its high accumulation in the soil and plants [56–58]. The Cr content of corn grains was between 0.34 and 1.59 ppm (as-is), which is considerably (approximately 5-folds) higher than the Cr levels of 0.07 and 0.28 ppm reported by Algül and Kara [59] for 18 corn samples collected from various cities in Turkey. The large difference on Cr level of corn grains between two studies could partly be due to different sample collection cites used in two studies and different sources (feed mills in the current study vs. human markets/groceries in the latter). Major plant parts that accumulate Cr have been reported as the root and leaves compared to the grains or fruits [60]. Therefore, accumulation of Cr in grains would unlikely to pose a threat to both human and animal health even under a heavy contamination [61]. NRC [62] has proposed a maximum daily Cr intake allowance of 100 ppm for pigs, horses, cattle, sheep, and 500 ppm for chickens. Therefore, theoretically, it does not seem possible that even consuming the corn with the highest Cr content in the current study would pose a problem for the animals.

Copper is an essential trace mineral that has many metabolic duties that directly affect health and therefore the traits of economic importance in animals. However, excessive amounts of dietary Cu can have detrimental effects on animals, with the liver being the major target organ, and also lead to a buildup of Cu in the environment [63, 64]. The Cu contents for corn grains reported in the current study were within the previously reported range of 0.8–10 ppm [6, 65, 66]. No adverse effects were reported from daily consumption of 10–12 mg of Cu in foods for humans with normal Cu homeostasis [67]. As another example, NRC [62] sets the maximum daily tolerable level of Cu to 40 ppm for dairy cattle. Therefore, even consumption of corn with the greatest Cu level (5.8 ppm) in the current study up to 2 kg for humans and 8 kg for cattle would not have a negative impact on the health of both species.

The specific function and dietary requirement level of Ni in humans and animals have not yet been identified. However, there are studies showing detrimental effects of high dietary Ni consumption such as immunotoxicity, neurotoxicity, and increased risk of developing different cancer types [68]. Ling and Leach [69] demonstrated that feeding 1,100 ppm Ni to broiler chicks resulted in anemia and increased mortality. Among the very few studies published, Oscar et al. [70] reported that the dietary level of 2.4 ppm Ni was adequate to produce satisfactory growth performance and carcass quality in broiler chickens. The range of Ni content (1.31–11.34 ppm, as-is) of corn grains obtained in the current study was comparable to those reported by Brizio et al. [50]. A maximum tolerance level of 100 ppm was established for cattle and sheep by NRC [62]; therefore, it seems that even the corn with the highest Ni content (11.34 ppm) in the current study can be tolerated by these animals at high dietary inclusion levels. The CV (54.07) for Ni content in corn grains was the greatest compared to all the other parameters studied in the current study. Therefore, more studies need to be directed toward the determination of the reason for such high variation in the Ni content of corn grains and toward understanding its role in the metabolism of animals.

Lead is considered as one of the most toxic heavy metals and has no reported essential role

Table 3. Correlations (R) between nutrients, and correlations between color scores (l*, a*, b*)[1] and nutrients for corn samples[2].

Item	DM	CP	EE	Ash	ADF	NDF	ADL	Starch	Ca	P
DM	–	–	–	–	–	–	–	–	–	–
CP	NS[3]	–	–	–	–	–	–	–	–	–
EE	−0.322*	NS	–	–	–	–	–	–	–	–
Ash	0.475***	0.472***	−0.281*	–	–	–	–	–	–	–
ADF	0.316*	NS	NS	0.306*	–	–	–	–	–	–
NDF	NS	NS	NS	0.292*	0.713***	–	–	–	–	–
ADL	0.562***	0.362**	NS	0.328*	0.313*	−0.143*	–	–	–	–
Starch	NS	NS	NS	NS	−0.323*	−0.362**	NS	–	–	–
Ca	NS	NS	0.401**	0.334*	NS	NS	NS	NS	–	–
P	0.345*	0.625***	NS	0.634***	NS	NS	0.415**	NS	NS	–
L*	NS[2]	NS	−0.258*	NS	NS	NS	NS	NS	NS	NS
a*	NS	NS	NS	NS	NS	0.316*	NS	NS	NS	NS
b*	−0.314*	NS	0.426**	−0.280*	NS	NS	−0.301*	NS	0.405**	NS

[1] L* = lightness, a* = redness, b* = yellowness.

[2] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$, double asterisk (**) for $P < 0.01$, and triple asterisk (***) for $P < 0.001$, respectively (n = 54).

[3] NS = Not significant.

for any biochemical and physiological functions in the living organism [71]. Bakalli et al. [72] found that 1 ppm dietary Pb resulted in a significant decrease on feed intake and depressing effect on overall live weight of broiler chickens. The mean Pb value obtained in the current study was approximately 50 times higher than those reported (0.01 ppm, as-is) by Brizio et al. [50] for 12 corn samples collected in Italy. One explanation for the large difference between studies is the source of samples taken. The samples used in the current study were gathered from feed mills; however, Brizio et al. [50] collected samples right after harvest that would be expected to have less time for getting Pb from the environment. On the other hand, the obtained maximum Pb level in the current study was very similar to those reported (0.98 ppm, as-is) by Lavado et al. [73]. The all Pb concentrations obtained in the current study exceeded the maximum acceptable level of 0.20 ppm defined by the EU regulation (no: 420/2011) for foodstuffs [74]. However, even the highest Pb level obtained in the current study was significantly lower than the 10 ppm (with 12% moisture) maximum tolerable Pb level posted by NRC for poultry feeds [39]. Therefore, the Pb levels obtained for corn samples used in the current study can be regarded as safe in regard to animal consumption.

Zinc is a trace mineral that involves in many metabolic processes including appetite control, immune system regulation, and growth and reproduction of animals [75, 76]. The Zn concentrations are within the range of reported values (16 to 130 ppm) by Demirkıran [77]. Considerably high dietary levels of Zn have been shown to be very well tolerated and pose no health concerns for both animals and humans [78]. For example, the toxicity threshold for dietary Zn was estimated to range from 500 to 1,500 ppm for dairy cattle [79]. Likewise, Southern and Baker [80] reported only a slight growth depression by feeding a relatively high level of Zn (2,000 ppm) to young chicks. Therefore, the obtained Zn levels for the corn samples in the current study would not pose any health concerns for animals.

Correlations Between Nutrients and Color Characteristics

In addition to determination of nutrient composition of the corn samples studied, another aim of the study was to evaluate correlations between nutrients, fatty acids, heavy metals, and DON concentrations. Although there were many significant correlations between investigated parameters at $P < 0.05$ and < 0.01 levels (Tables 3–9), only correlations determined to be statistically significant at $P < 0.001$, which are of practical importance, are discussed in the current study. Moreover, the correlations at $P < 0.001$ significance having R values between

Table 4. Correlations (*R*) between fatty acids and color scores (l*, a*, b*)[1] for corn samples[2].

Item	C16:0	C18:0	C18:1	C18:2 n-6	C18:3 n-3
C16:0	–	NS[3]	NS	−0.445***	NS
C18:0	–	–	NS	−0.325*	NS
C18:1	–	–	–	−0.868***	NS
C18:2 n-6	–	–	–	–	NS
C18:3 n-3	–	–	–	–	–
L*	−0.275*	NS	0.347**	NS	NS
a*	NS	NS	NS	NS	NS
b*	NS	NS	NS	NS	−0.267*

[1] L* = lightness, a* = redness, b* = yellowness.

[2] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$, double asterisk (**) for $P < 0.01$, and triple asterisk (***) for $P < 0.001$, respectively (n = 54).

[3] NS = Not significant.

Table 5. Correlations (*R*) between heavy metals and color characteristics (L*, a*, b*)[1] for corn samples[2].

	Cd	Cr	Cu	Ni	Pb	Zn
Cd	–	NS[3]	0.498***	NS	0.639***	NS
Cr	–	–	NS	0.318*	NS	0.409**
Cu	–	–	–	NS	0.641***	0.484***
Ni	–	–	–	–	NS	NS
Pb	–	–	–	–	–	0.459***
Zn	–	–	–	–	–	–
L*	NS	NS	NS	NS	NS	NS
a*	NS	NS	NS	0.341*	NS	NS
b*	NS	NS	NS	NS	NS	0.291*

[1] L* = lightness, a* = redness, b* = yellowness.

[2] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$, double asterisk (**) for $P < 0.01$, and triple asterisk (***) for $P < 0.001$, respectively (n = 50).

[3] NS = Not significant.

Table 6. Correlations (*R*) between nutrients and heavy metals for corn samples[1].

	Cd	Cr	Cu	Ni	Pb	Zn
DM	NS[2]	NS	−0.280*	NS	NS	−0.342*
CP	NS	NS	NS	NS	NS	NS
EE	NS	NS	0.403**	−0.293*	0.290*	0.439**
Ash	NS	NS	NS	NS	NS	NS
ADF	NS	NS	NS	NS	NS	NS
NDF	NS	NS	NS	NS	NS	NS
ADL	NS	NS	NS	NS	NS	−0.307*
Starch	NS	NS	NS	NS	NS	NS
Ca	NS	0.528***	0.336*	NS	0.317*	0.672***
P	NS	NS	NS	NS	NS	NS

[1] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$, double asterisk (**) for $P < 0.01$, and triple asterisk (***) for $P < 0.001$, respectively (n = 50).

[2] NS = Not significant.

± 0.45 and 0.65 were classified as "moderate" and between ± 0.66 and 0.85 were classified as "high." The prediction equations calculated from significant correlations ($P < 0.01$) are given in Table 10. Significant correlations between nutrient composition and color characteristics of corn samples are provided in Table 3. The highest correlation was between NDF and ADF (0.713) followed by moderate correlations between P and ash (0.634), and P and CP (0.625). Similarly, Wang and Daun [81] showed that there was a very high linear correlation

Table 7. Correlations (*R*) between fatty acid composition, nutrients and heavy metals for corn samples[1].

	C16:0	C18:0	C18:1	C18:2 n-6	C18:3 n-3
DM	NS[2]	NS	NS	NS	0.416**
CP	−0.288*	NS	NS	NS	NS
EE	0.287*	NS	NS	NS	−0.282*
Ash	−0.390**	NS	NS	NS	NS
ADF	NS	NS	NS	NS	NS
NDF	NS	0.288*	NS	NS	NS
ADL	NS	NS	NS	NS	NS
Starch	NS	−0.379**	NS	NS	NS
Ca	NS	NS	NS	NS	−0.495***
P	NS	NS	NS	NS	NS
Cd	NS	NS	NS	NS	NS
Cr	NS	NS	NS	NS	NS
Cu	NS	NS	NS	NS	−0.476***
Ni	NS	NS	NS	0.280*	NS
Pb	NS	NS	NS	NS	−0.354*
Zn	NS	NS	NS	NS	−0.362**

[1] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$, double asterisk (**) for $P < 0.01$, and triple asterisk (***) for $P < 0.001$, respectively. n = 54 for correlations between fatty acids and nutrients. n = 50 for correlations between fatty acids and heavy metals.

[2] NS = Not significant.

Table 8. Occurrence of deoxynivalenol (DON) and its concentration in DON positive corn grains[1].

		DON concentrations in positive samples (mg/kg)			
	Positive samples/total sample (%)	Minimum	Maximum	Median	Mean
DON	26/49 (53%)	0.040	0.725	0.100	0.158

[1] n = 49.

Table 9. Correlations (*R*) between deoxynivalenol (DON) level, nutrients, heavy metals, fatty acids, and color characteristics for corn samples[1].

	Nutrients									
Item	DM	CP	EE	Ash	ADF	NDF	ADL	Starch	Ca	P
DON	NS[2]	0.399*	NS	NS	NS	NS	NS	NS	NS	NS
	Heavy metals									
	Cd	Cr	Cu	Ni	Pb	Zn				
DON	NS	−0.430*	NS	NS	NS	NS				
	Fatty acids and color characteristics									
	C16:0	C18:0	C18:1	C18:2	C18:3	L*	a*	b*		
DON	NS	NS	NS	NS	NS	−0.425*	NS	NS		

[1] Significant correlations are expressed using a single asterisk (*) for $P < 0.05$.

[2] NS = Not significant.

between ADF and NDF in alfalfa hay. Since both ADF and NDF are the representation of fiber in plant tissues with hemicelluloses being the only difference, the more ADF in corn, the greater the NDF content, as would be expected. Phosphorus is the most abundant mineral in corn following K content as a macro-mineral [6] and ash represents the total mineral content; therefore, it is not surprising to obtain a significant correlation between ash and P in the current study. Phosphorus and CP correlated moderately, which is contrary to the results observed in Japonica rice and field beans by others [82, 83]. However, those two studies correlated phytate P or phytic acid with protein content instead of total P as in the current study. Moreover, the difference in results between studies could be also, at least partially, due to the different

Table 10. Equations generated from single Linear Regression between variables that have significant correlations ($P < 0.01$)[1].

Correlations	Units	P-value	R	R^2	Equation
CP–Ash	%–%	***	0.472	0.222	CP (%) = (2.662 × Ash) + 4.716
EE–b*	%–score	**	0.426	0.183	EE (%) = (0.081 × b*) + 0.86
Ash–DM	%–%	***	0.475	0.225	Ash (%) = (0.045 × DM) – 2.941
ADF–NDF	%–%	***	0.713	0.508	ADF (%) = (0.2 × NDF) + 0.686
ADL–DM	%–%	***	0.562	0.316	ADL (%) = (0.162 × DM) – 13.37
ADL–CP	%–%	**	0.362	0.130	ADL (%) = (0.188 × CP) – 0.485
Starch–NDF	%–%	**	– 0.362	0.131	Starch (%) = (−0.547 × NDF) + 73.50
C16:0–Ash	%–%	**	– 0.390	0.152	C16:0 (%) = (−4.100 × Ash) + 15.26
C 18:0–Starch	%–%	**	– 0.379	0.143	C18:0 (%) = (−0.122 × Starch) + 9.869
C18:1–L*	%–score	**	0.347	0.120	C18:1 (%) = (0.771 × L*) – 38.29
C18:2–C16:0	%–%	***	– 0.445	0.198	C18:2 (%) = (−1.029 × C16:0) + 68.96
C18:2–C18:1	%–%	***	– 0.868	0.754	C18:2 (%) = (−0.990 × C18:1) + 86.16
C 18:3–DM	%–%	**	0.416	0.174	C18:3 (%) = (0.066 × DM) – 4.700
C18:3–Ca	%–%	***	– 0.495	0.245	C18:3 (%) = (−3.186 × Ca) + 1.456
C18:3–Cu	%–ppm	***	– 0.476	0.226	C18:3 (%) = (−0.107 × Cu) + 1.463
C18:3–Pb	%–ppm	*	– 0.354	0.125	C18:3 (%) = (−0.404 × Pb) + 1.396
C18:3–Zn	%–ppm	**	– 0.362	0.131	C18:3 (%) = (−0.007 × Zn) + 1.400
Ca–EE	%–%	**	0.401	0.161	Ca (%) = (0.023 × EE) + 0.007
Ca–b*	%–score	**	0.405	0.164	Ca (%) = (0.004 × b*) – 0.046
P–ADL	%–%	**	0.415	0.172	P (%) = (0.045 × ADL) + 0.186
P–Ash	%–%	***	0.634	0.402	P (%) = (0.206 × Ash) + 0.009
P–CP	%–%	***	0.625	0.391	P (%) = (0.036 × CP) – 0.043
Cd–Cu	ppm–ppm	***	0.498	0.247	Cd (ppm) = (0.010 × Cu) + 0.007
Cr–Ca	ppm–%	***	0.528	0.278	Cr (ppm) = (4.785 × Ca) + 0.369
Cr–Zn	ppm–ppm	**	0.409	0.167	Cr (ppm) = (0.012 × Zn) + 0.434
Cu–EE	ppm–%	**	0.403	0.162	Cu (ppm) = (0.677 × EE) + 0.366
Pb–Cd	ppm–ppm	***	0.639	0.409	Pb (ppm) = (5.847 × Cd) + 0.303
Pb–Cu	ppm–ppm	***	0.641	0.411	Pb (ppm) = (0.126 × Cu) + 0.184
Pb–Zn	ppm–ppm	***	0.459	0.210	Pb (ppm) = (0.008 × Zn) + 0.273
Zn–EE	ppm–%	**	0.439	0.192	Zn (ppm) = (8.006 × EE) + 2.000
Zn–Ca	ppm–%	***	0.672	0.452	Zn (ppm) = (208.0 × Ca) + 10.31
Zn–Cu	ppm–ppm	***	0.484	0.234	Zn (ppm) = (5.245 × Cu) + 14.25

[1] Double asterisk (**) indicates $P < 0.01$, and triple asterisk (***) indicates $P < 0.001$, respectively.

plant species evaluated. A moderate correlation was found between ADL and DM (0.562). This result agrees with those of Wilkinson et al. [84] in which a low correlation was found between ADL and DM for coastal bermudagrass. Although crude ash was significantly correlated to DM (0.475) and CP (0.472), low R^2 values (0.225 and 0.222, respectively) obtained suggest that the calculated regression equations (Table 10) would have limited practical significance in the field for these two correlations.

Correlations Between Fatty Acids and Color Characteristics

Significant correlations between fatty acids and color characteristics of corn samples are provided in Table 4. Notably, the negative correlation (−0.868) between C18:2 and C18:1 was the highest correlation with a relatively high R^2 (0.754) in the current study. Similarly, Green [85] reported a very high (−0.97) negative correlation between C18:2 and C18:1 content in flax seed oil. In contrast, Kitta et al. [86] and Saastamoinen et al. [87] reported significant positive correlations between C18:2 and C18:1 content in rice and oat, respectively. Although there was a significant correlation between C18:2 and C16:0, its relatively low R^2 (0.198) decreases its practical importance.

Correlations Between Heavy Metals and Color Characteristics

Significant correlations between heavy metal composition and color characteristics of corn samples are provided in Table 5. The low R^2 values (0.247 and 0.234) obtained for the significant correlations between Cd and Cu and between Zn and Cu suggest that the equations generated have limited practical importance. However, Cu ($R^2 = 0.411$) and Cd ($R^2 = 0.409$) correlated well with Pb content in corn grains, indicating that these two heavy metals might be used for predicting the Pb levels in corn samples. Similar significant positive correlations have also been reported between the Cd and Pb levels in asparagus bean, *Brassica Chinensis*, rice, wild rice, and wheat [88–91]. The authors suggested that Pb could enhance the activity of plants to accumulate more Cd. This phenomenon seems to be also true for the corn grains in the current study. Moreover, our findings may also support the statement that plants are mostly contaminated by more than one metal in the field [92]. In wild rice, Pip [88] reported no correlation between Pb and Cu levels, which conflicted with the current study. Although the correlation between Zn and Pb was moderate, the low R^2 (0.210) again suggested that the prediction equation generated would be of limited practical importance. However, Lu et al. [93] reported a significant positive correlation between Pn and Zn concentrations in soils collected in China. Therefore, the phenomena of multiple heavy metal contamination may also be true for Pb and Zn levels in both soil and corn samples studied in the current study [92].

Correlations Between Nutrients, Heavy Metals, and Fatty Acids

Although four significant correlations ($P < 0.01$) existed between nutrients and heavy metals, only the correlation between Zn and Ca would be of practical importance with a relatively high R (0.672) and R^2 (0.452) value in the current study (Table 6). Among fatty acids, nutrients, and heavy metals, the strongest correlations were between Ca and C18:3 ($R = -0.495$), and Cu and C18:3 ($R = -0.476$) (Table 7).

Deoxynivalenol Occurrence and Correlations for Deoxynivalenol

Deoxynivalenol was found in 26 out of the 49 corn samples tested (53%), with all DON positive samples having less than 1 ppm in the current study (Table 8). Ransom et al. [94] reported DON as the most commonly detected mycotoxin with a 17% occurrence rate in 94 corn samples from North Dakota in USA. The result of the current study agreed with Oliveira et al. [95], who reported a frequency of DON occurrence in 48% of corn samples (n = 148), with a maximum level of 1 ppm in Brazil. However, the DON occurrence in the current study is lower than a previous study conducted in Turkey reporting 80% DON positivity in 20 corn samples [96]. The highest level of DON found in the current study is below the current advisory maximum level for use in feedstuffs of 10 ppm for poultry and ruminants by FDA [97]. Moreover, even the highest DON concentration (0.725 ppm) obtained in the current study was lower than the EU regulation limit of 1.75 ppm DON posted for unprocessed corn [98]. Nevertheless, while even the highest DON level can be regarded as safe for animals and humans, the high occurrence rate obtained in the current study implies a requirement for a close monitoring of DON levels in corn. No correlation of practical importance was found between DON and nutrients, heavy metals, and fatty acids in the current study (Table 9).

CONCLUSIONS AND APPLICATIONS

1. The variation was especially high for Ca, P, and EE among the nutrients of corn, which may suggest a need to more close monitoring of these nutrients to optimize its utilization by animals.
2. The color characteristics (L*, a*, b*) were not a good predictor of any nutrients, heavy metals, and DON concentration in corn grains.
3. Although all samples exceeded the maximum tolerable limit set for Pb by EU for humans, they were within the safe limit for animals. All the other analyzed heavy metal (Cd, Cr, Cu, Ni, and Zn) levels were below the maximum tolerable levels set by different agencies both for humans and animals.
4. The results of the current study showed that the significant correlations found between nutrients, minerals, and heavy met-

als can be used to predict related contents such as C18:2, ADF, Zn, Pb, and P in corn grains.

5. None of the DON positive samples had concentrations above maximum tolerable levels set by FDA and EU.

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