Animal Nutrition 15 (2023) 1–9

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Feasibility of feeding cadmium accumulator maize (*Zea mays* L.) to beef cattle: Discovering a strategy for eliminating phytoremediation residues

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ARTICLE INFO

Article history:
Received 28 September 2022
Received in revised form
16 June 2023
Accepted 27 June 2023
Available online 20 July 2023

Keywords:
Beef cattle
Cadmium
Performance
Health

ABSTRACT

Eco-friendly and efficient strategies for eliminating cadmium (Cd) phytoremediation plant residues are needed. The present study investigated the feasibility of feeding Cd accumulator maize to beef cattle. In total, 20 cattle at 6 months of age were selected and randomly allocated into two groups fed with 85.82% (fresh basis) Cd accumulator maize (CAM) or normal maize (control [Con]) silage diets for 107 d. Feeding CAM did not affect the body weight ($P = 0.24$), while it decreased feed intake and increased feed efficiency of beef cattle ($P < 0.01$). Feeding CAM increased serum concentrations of immunoglobulin A and G, complement 3 and 4, blood urea nitrogen, and low-density lipoprotein cholesterol, decreased serum concentrations of interleukin-6 and lipopolysaccharide ($P < 0.05$), and caused wider lumens in the renal tubules. The Cd residue in meat was 7 μg/kg beyond the restriction for human food. In the muscle, the unsaturated fatty acids (t11C18:1 and C20:4), Lys, Arg, Pro, and Cys were decreased, while the saturated fatty acids (C10:0, C12:0, and C17:0) and Leu were increased ($P < 0.05$). Therefore, at the current feeding level, phytoremediation maize increased the feed efficiency of beef cattle, but did present risks to cattle health and production safety, and decreased the meat nutrition and flavor. Further research must be performed to determine whether a lower proper dose of phytoremediation maize and an appropriate feeding period may be possible to ensure no risk to cattle health and the supply of safe meat for humans.

1. Introduction

Cadmium (Cd) and its compounds are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC). Various types of cancers such as breast, lung, prostate, pancreatic, and kidney cancers and organ system toxicities, including bone, urinary, reproductive, central nervous, respiratory and cardiovascular toxicities are associated with environmental exposure to Cd (Rafati Rahimzadeh et al., 2017).

In China, Cd is a major pollution affecting agricultural soil quality (MEE, 2019). Phytoremediation is an economically feasible and eco-friendly rehabilitation strategy for the remediation of heavy metal-contaminated farmland. Aliyu and Adamu (2014) reported that maize (*Zea mays* L.) had a high tolerance and uptake ability to various heavy metals, making it a promising phytoextraction plant. With a high bioaccumulation effect for Cd, maize is regarded as a potential Cd hyperaccumulator (Aladesanmi et al., 2019). Phytoremediation causes secondary environmental pollution by producing large amounts of Cd-rich plant residues (Ali et al., 2013). Xie et al. (2020) found that straw removal during rice harvesting contributed 66% to 78% of the Cd output for Cd-polluted

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.06.012

paddy soil in Hunan Province, while the opposite was true under a straw returning scenario. Thus, a Cd hyperaccumulator, in which phytoremediation residues can be treated in an eco-friendly and efficient manner, is urgently needed for a feasible strategy of Cd phytoremediation.

Whole-plant maize is a high-quality feedstuff that provides high energy (mainly from starch in the kernel fraction) along with physically effective neutral detergent fiber (provided by the stover fraction) concurrently (Ferraretto et al., 2018). The large-scale development of the dairy and beef industry in China has increased the demand for forage, so feeding Cd accumulator maize (CAM) to beef cattle might be a strategy for eliminating CAM residues; however, the use of CAM for animal feedstock raises food safety risk concerns. Beef cattle are special animals containing a multichambered stomach system, which can degrade toxic compounds contained in feed by a variety of microorganisms colonized in the rumen (Loh et al., 2020). A recent study revealed that *Paracoccus* sp. LZ-G1 isolated from cow feces could degrade cellulose and adsorb Cd (Guo et al., 2021), suggesting the possibility of crosstalk between Cd and the gut microorganisms of cattle.

Therefore, it is very important to verify whether CAM can be fed to beef cattle as a feedstuff with less negative effects on growth and health, while lowering Cd contamination in their products. In this experiment, the CAM was fed to beef cattle after ensiling, and the growth and production performance, health status, and Cd residue in animals were measured to evaluate the feasibility of feeding phytoremediation maize to beef cattle.

2. Materials and methods

2.1. Animal ethics statement

This experiment was conducted at the National Origin Farm of Xiangxi Yellow Cattle, Huayuan County, Hunan Province, China. All experimental protocols and procedures used in this study were permitted by the Animal Care and Use Committee of Zhejiang University (ZJU20230094) and all animal experiments complied with the ARRIVE guidelines.

2.2. Animals, feed, and experimental design

In total, 10 male and 10 female healthy crossbred (Angus bull × Xiangxi local cattle) weaned cattle (body weight = 79.63 ± 4.183 kg; mean ± SE) at 6 months of age were selected from a paternal half-sib family, and randomly allocated into two groups (control group [Con] and Cd accumulator maize group [CAM]) with five male and five female cattle in each group. The experimental cattle were housed in two open-sided naturally well-ventilated barns (8 m × 7.5 m × 1.5 m) by group. Within each group, cattle were separated by gender with railings. The experimental diet for both groups were mixed daily and provided to the cattle as a total mixed ration. The ingredients and chemical compositions of the total mixed ration are listed in Table 1. The corn silage used for CAM was made by the whole plant of a Cd accumulator maize species (Yuqing 23), which was planted in the most excessive Cd soil (soil Cd concentration = 4.76 ± 0.930 mg/kg, pH = 5.58 ± 0.196, mean ± SE) in Liuyang City, Hunan Province, China. The corn silage used for the Con was made from whole-plant maize planted in Xiangxi Tujia and Miao Autonomous Prefecture with a normal Cd concentration in the soil (soil Cd concentration = 0.12 ± 0.017 mg/kg, pH = 5.59 ± 0.098, mean ± SE) under the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land in China (GB 15618-2018) (Cd concentration ≤ 0.3 mg/kg, pH ≤ 7.5) (MEE, 2018). The Cd concentrations of the Con and CAM diets were 0.72 and 6.74 mg/kg, respectively. The orts were removed once daily prior to the morning feeding and new feed was delivered 2 times daily at 07:00 and 17:00. Ten cattle within one group shared one feed trough. All cattle had free access to water. The feeding experiment was conducted for 107 d.

Table 1
Ingredients and chemical composition of the total mixed ration administered to the cattle of Con and CAM.

Item	Con	CAM	SEM	*P*-value
Ingredients (fresh basis, %)				
Straw	2.84	2.84	–	–
Corn silage	85.82	85.82	–	–
Pelleted concentrate[1]	11.34	11.34	–	–
Total	100	100	–	–
Chemical composition[2] (DM basis, %)				
GE, MJ/kg	12.00	13.36	0.406	0.09
DM (fresh basis)	39.97	29.16	1.330	< 0.01
CP	10.79	11.45	0.292	0.30
EE	11.00	13.00	0.730	0.20
NDF	49.33	50.67	1.732	0.74
ADF	25.00	25.33	1.195	0.91
Ca	0.62	0.62	0.028	0.95
P	0.42	0.37	0.022	0.32
Cd, mg/kg	0.72	6.74	1.516	< 0.01

Con = control; CAM = Cd accumulator maize.
[1] Pelleted concentrate was purchased from Purina with the following chemical composition (fresh basis, %): crude protein ≥20.0, crude fiber ≤10.0, ash ≤10.0, 0.5 ≤ Ca ≤1.5, P ≥ 0.45, 0.25 ≤ sodium chloride ≤1.0, Lys ≥1.
[2] GE = gross energy; DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; Ca = calcium; P = phosphorus; Cd = cadmium.

2.3. Sample collection and performance measurement

The feed intake of each group was recorded every 15 d. Body weight (BW) and body size (including body height, body side length, hip cross height, cannon bone circumference, bust, abdominal circumference, and testicular circumference) of cattle were measured on d 0 and 107 of the feeding trial. On d 107, all cattle were sacrificed by electrical stunning and exsanguination in the slaughter room of the National Origin Farm of Xiangxi Yellow Cattle after fasting for 24 h.

Subsamples of daily feed were used for chemical analysis to determine nutrition intake. Feed samples were dried in a forced-air oven at 65 °C for 48 h and ground to allow passage through a 1-mm sieve (HK-08 A ground mill, Xu Lang Machinery, Guangzhou, China). Then, they were stored in sealed plastic containers at 4 °C until analysis.

Approximately 10 mL of blood was collected from the jugular vein into a heparinized vacutainer tube on d 0, 40, 70 and 107 of the feeding trials before morning feeding. After standing for 30 min at room temperature, the blood samples were centrifuged at 1,500 × *g* at 4 °C for 15 min to obtain the serum and stored at −80 °C until further analysis.

Muscle (right-side longissimus dorsi muscle), brain, and visceral (heart, liver, spleen, lung, and kidney) samples were taken immediately after sacrifice, two pieces were taken for each organ; one piece was fixed in 10% formaldehyde solution for morphological and pathological observation, and the other was stored at −20 °C for Cd content measurement.

The carcasses were split in half, and the longissimus dorsi muscle was cut between the 12th and 13th ribs. The loin-eye area was measured by a backfat tester (EXAGO, DAOU Technology, France). Approximately 2 kg of the right-side longissimus dorsi muscle was taken at 24 h postmortem for measurement of meat quality traits.

2.4. Feed nutrition analysis

Gross energy (GE) was determined using a calorimeter (Kaiyuan, Changsha, China). The dry matter (DM; method 924.05; AOAC, 1990), crude protein (CP; method 988.05; AOAC, 1990), ether extract (EE; method 954.02; AOAC, 1990), calcium (Ca; method 935.13; AOAC, 1990) and phosphorus (P; method 964.06; AOAC 1990) of feed samples were determined. The neutral detergent fiber (NDF; with heat-stable amylase and sodium sulfite) and acid detergent fiber (ADF) contents were determined using Ankom filter bags and fiber analyzer equipment (Ankom Technology, NY, USA) following the method of Van Soest et al. (1991).

2.5. Blood analysis

The concentrations of glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and serum amyloid-A (SAA) were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using a Hitachi 7020 autobiochemistry instrument (Hitachi, Tokyo, Japan). The concentrations of immunoglobulin M (IgM), A (IgA), and G (IgG), complement 3 (C3) and 4 (C4), lipopolysaccharide (LPS), interleukin-1β (IL-1β), -6 (IL-6), and -22 (IL-22), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and insulin growth factor-1 (IGF-1) were determined with commercial ELISA kits (mlbio, Shanghai, China) using a microplate reader (RT-6100, Rayto, Shenzhen, China).

2.6. Measurements of meat quality traits

The initial and final pH were reflected by the pH at 30 min and 24 h postmortem, respectively, which were measured with a previously calibrated pH meter (PHS−3C, Shanghai, China). Meat color measurements were obtained from an average of 3 readings across the surface over randomly identified locations using a flesh colorimeter (SCQ-1A, Beijing, China). One piece (1 cm × 1 cm × 2 cm) was cut from each sample for the determination of drip-loss following the method of Honikel (1998). Cook loss was determined following the method of Nasr et al. (2017). After the core temperature of the cooked meat samples cooled to 0−4 °C, 3 pieces longer than 2.5 cm along the direction parallel to the muscle fibers were taken by a circular borehole sampler to measure the shear force immediately with a muscle tenderness determination device (CLM-3B, Tenovo International Co., Limited, Beijing, China).

2.7. Muscle fatty acid analysis

Muscle samples (approximately 1 g) were thawed and minced with water (approximately 1 mL). Fat was extracted with 6 drops of HCl (6 mol/L) and 4 mL chloroform-isopropanol (9:1; v/v) solution. After vacuum drying, the fat extract was mixed with 2 mL boron trifluoride ether-methanol solution (1:3; v/v) and esterified in a water bath at 80 °C. After cooling, 4 mL water and n-hexane (1:1; v/v) were added, and the supernatant was passed through a 0.22-μm filter membrane for GC−MS analysis. The fatty acid compositions of the muscle samples were determined with a gas chromatography−mass spectrometry (6890 N GC-5973MSD, Agilent Technologies Inc., California, USA).

2.8. Muscle amino acid analysis

Dried and defatted muscle samples (approximately 0.1 g) were hydrolyzed in a vacuum-sealed hydrolysis tube containing 4 mL HCl (6 mol/L) in an oven at 110 °C for 24 h after flushing with nitrogen. After cooling, samples were diluted to 100 mL, and a 2-mL subsample was deacidified at 60 °C. Then, 2 mL HCl (0.02 mol/L) was added to the sample, and the sample was mixed with a vortex and filtered through a 0.22-μm membrane filter. The amino acid profile of each sample was determined by an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). The detection wavelengths of Pro and other amino acids were 440 and 570 nm, respectively.

2.9. Cadmium analysis

Approximately 1 g of sample was digested with 3 mL of HNO_3 in a digestion tank according to the following procedure: the temperature was increased to 120 °C within 5 min and kept for 3 min, then the temperature was increased to 180 °C within 5 min and kept for 8 min. After digestion, all solutions were transferred into a 10-mL colorimetric tube and diluted to 10 mL with 5% HCl. The Cd concentration was detected by inductive coupled plasma-mass spectrometry (7700x, Agilent Technologies Inc., California, USA) with deionized water used as a blank control.

2.10. Morphological measurements of tissues

Specimens of heart, liver, spleen, lung, kidney, and muscle were fixed in 10% formaldehyde solution for morphological measurements. After being dehydrated, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin, the specimens were examined under an optical microscope (80i, Nikon, Tokyo, Japan).

2.11. Statistical analyses

The results were analyzed under a completely randomized study design. One male fed CAM had growth arrest during the experiment; thus, the data of this animal were removed. Since the growth rates of male and female cattle were similar during the feeding trial, the gender effect was not considered in the present study.

All experimental data were analyzed by IBM SPSS statistics 25 software (IBM Corp, NY, USA). Mixed linear model was used to test variations among different groups; when each animal was used as an experimental unit, the treatment was considered a fixed effect, and individual animal was considered a random effect. Significance was defined as $P < 0.05$ and highly significant was defined as $P < 0.01$.

3. Results

3.1. Growth performance and organ weights

During the feeding trial, the average dry matter intake (ADMI) of CAM cattle was 3.11 kg/d, which was significantly lower than that of Con cattle, 4.37 kg/d ($P < 0.01$, Table 2). Along with the ADMI, the intakes of CP and NDF of CAM cattle were significantly lower than those of Con cattle, and the Cd intake of CAM cattle was significantly higher than that of Con cattle ($P < 0.01$, Table 2). The initial (d 0) and end BW (d 107) of cattle were not different between the two groups ($P > 0.05$, Table 2). The average daily gain (ADG) did not differ between the two groups ($P = 0.20$), while CAM cattle showed a higher feed efficiency than Con cattle ($P < 0.01$, Table 2). No difference was observed in body size between the two groups ($P > 0.05$, Table 3). Among the visceral organs, the weight of the lungs of CAM cattle was significantly higher than that of Con cattle ($P = 0.05$) and the proportions of the weights of the visceral organs to BW did not differ between the two groups ($P > 0.05$, Table 2).

Table 2
Growth performance and visceral organ weights of cattle in Con and CAM.

Item[1]	Con	CAM	SEM	*P*-value
Performance				
ADMI, kg/d	4.37	3.11	0.191	< 0.01
CP intake, kg/d	0.47	0.36	0.018	< 0.01
NDF intake, kg/d	2.15	1.58	0.089	< 0.01
Cd intake, mg/d	3.14	20.97	2.508	< 0.01
Initial BW (d 0), kg	77.70	81.78	4.183	0.64
End BW (d 107), kg	129.45	145.00	7.395	0.24
ADG, kg/d	0.48	0.59	0.041	0.20
F:G ratio	10.47	5.66	0.976	< 0.01
Weight of visceral organs				
Heart, kg	0.56	0.63	0.031	0.27
Liver, kg	1.95	2.31	0.117	0.13
Spleen, kg	0.36	0.33	0.032	0.68
Lung, kg	0.94	1.18	0.063	0.05
Kidney, kg	0.35	0.42	0.020	0.06
Proportion to BW				
Heart, %	0.44	0.44	0.011	0.74
Liver, %	1.51	1.60	0.031	0.11
Spleen, %	0.27	0.22	0.018	0.18
Lung, %	0.73	0.83	0.031	0.10
Kidney, %	0.27	0.30	0.008	0.14

Con = control; CAM = Cd accumulator maize.
[1] ADMI = average dry matter intake; CP = crude protein; NDF = neutral detergent fiber; BW = body weight; ADG = average daily gain; F:G ratio = feed efficiency.

Table 3
Body size of cattle in Con and CAM at the beginning (d 0) and end of the study (d 107).

Item	Con	CAM	SEM	*P*-value
d 0				
Body height, cm	84.50	83.44	1.195	0.67
Body side length, cm	93.90	91.67	1.938	0.58
Hip cross height, cm	88.20	87.56	1.302	0.81
Cannon bone circumference, cm	12.40	11.50	0.799	0.61
Bust, cm	103.30	102.33	1.649	0.78
Abdominal circumference, cm	121.50	128.00	2.555	0.21
Testicular circumference, cm	12.40	11.50	0.799	0.61
d 107				
Body height, cm	96.80	97.89	1.423	0.47
Body side length, cm	108.50	107.00	2.433	0.95
Hip cross height, cm	101.40	102.22	1.279	0.33
Cannon bone circumference, cm	13.80	14.22	0.342	0.51
Bust, cm	125.50	130.00	2.368	0.15
Abdominal circumference, cm	145.60	147.44	2.970	0.26
Testicular circumference, cm	18.00	18.25	1.328	0.88

Con = control; CAM = Cd accumulator maize.

3.2. Residues of cadmium in visceral organs, brain and muscle

Cd had different residue levels in visceral organs and muscle, with the brain having the least Cd residue (4.26 and 5.78 μg/kg in Con and CAM cattle, respectively) and the kidney having the highest Cd residue (506.42 and 2149.14 μg/kg in Con and CAM cattle, respectively) followed by the liver (144.17 and 721.60 μg/kg in Con and CAM cattle, respectively, Fig. 1). Cd residues in the heart, liver, lung, kidney, and muscle in the CAM group were significantly higher than those in the Con group, which were 1.62, 5.01, 1.85, 4.24, and 2.31 times, respectively ($P < 0.01$, Fig. 1). No significant difference in Cd residues was observed in the spleen ($P = 0.15$) or brain ($P = 0.08$) between the two groups (Fig. 1).

3.3. Histology of visceral organs and muscle

In the kidney, wider lumens were observed in the renal tubules of CAM cattle (Fig. 2). No obvious difference was observed in the histopathological examination of the heart, spleen, lung, and muscle between the Con and CAM groups (Fig. 2).

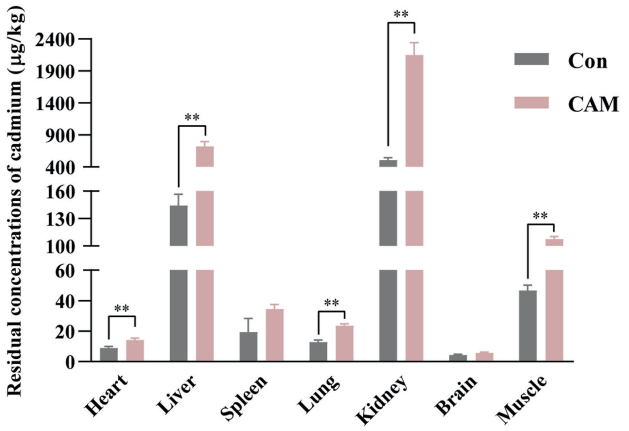

Fig. 1. Cadmium (Cd) residues in the visceral organs, brain, and muscle of Con and CAM cattle at the end of the feeding trial. Double asterisks (**) represents $P < 0.01$. Con = control; CAM = Cd accumulator maize.

3.4. Serum variables

The serum physiological, inflammatory, and immunological variables are shown in Table 4. After consuming the CAM diet, no serum physiological variables were affected on d 40. On d 107, the concentrations of BUN ($P = 0.03$) and LDL-C ($P < 0.01$) were significantly increased. For the inflammatory and immunological variables, compared to Con cattle, the concentration of C3 was significantly increased on d 40 ($P = 0.04$) and d 70 ($P = 0.02$), and the concentration of C4 was significantly increased on d 107 in CAM cattle ($P = 0.04$). Also, the concentration of IgA was significantly increased on d 107 ($P < 0.01$), and the concentration of IgG was significantly increased on d 40 ($P = 0.03$) and d 107 ($P = 0.03$) in CAM cattle. In addition, the concentration of IL-6 was significantly decreased on d 107 ($P < 0.01$) in CAM cattle, and the serum concentration of LPS was significantly lower in CAM cattle than in Con cattle on both d 70 ($P = 0.04$) and d 107 ($P = 0.03$).

3.5. Meat quality and nutrition

The meat performance and quality variables are shown in Table 5. No difference was observed for all variables between the two groups.

The results regarding the amino acid composition of muscle in Con and CAM cattle are summarized in Table 6. The concentrations of essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (TAA) were 8.786, 12.270, and 21.056 g/100 g in Con cattle and 8.713, 12.096, and 20.809 g/100 g in CAM cattle, respectively. Glu had the highest concentration (3.524 g/100 g in Con cattle and 3.551 g/100 g in CAM cattle), and Cys had the lowest concentration (0.083 g/100 g in Con cattle and 0.079 g/100 g in CAM cattle). Among these amino acids, the concentrations of Lys ($P < 0.01$), Arg ($P < 0.01$), Pro ($P = 0.03$), and Cys ($P = 0.01$) were significantly lower in the CAM group and the concentration of Leu was significantly higher in the CAM group than in the Con group ($P = 0.03$).

The muscle contained 47.70% saturated fatty acids (SFA), 41.19% monounsaturated fatty acids (MUFA), and 11.10% polyunsaturated fatty acids (PUFA) in Con cattle and 47.88% SFA, 41.03% MUFA, and 11.08% PUFA in CAM cattle. Among the fatty acids, C18:1 (36.21% in Con cattle and 36.23% in CAM cattle) had the highest proportion, followed by C16:0 (24.27% in Con cattle and 24.26% in CAM cattle)

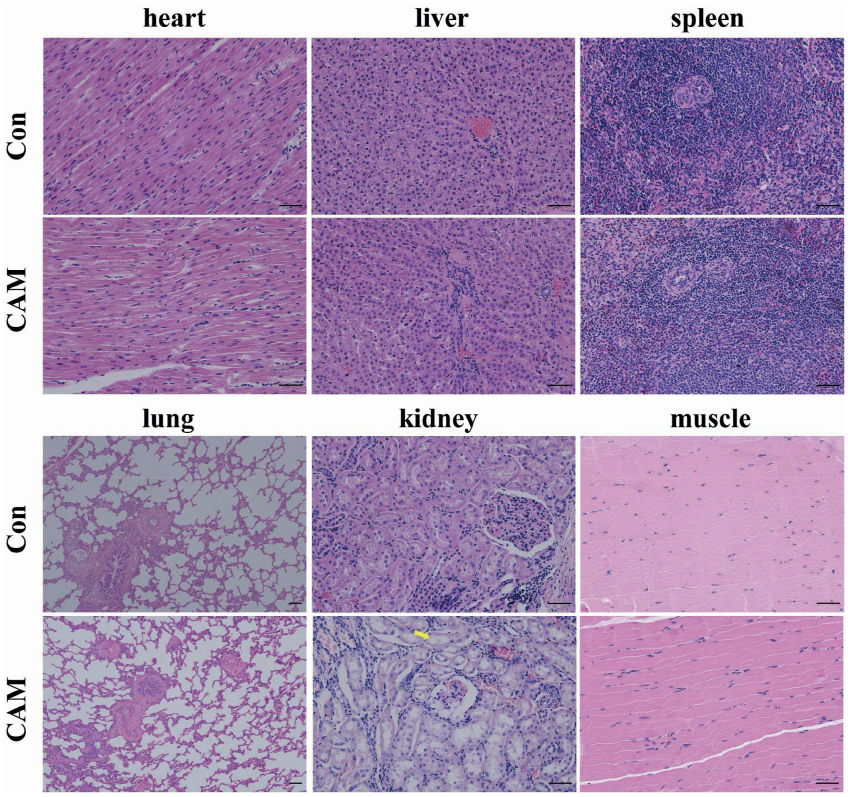

Fig. 2. Photomicrograph of heart, liver, spleen, lung, kidney and muscle of cattle in Con and CAM. Wider lumens were observed in the renal tubules in CAM, and no significant difference was found in the morphological structure of other tissues between two groups. Scale bar = 50 μm. Con = control; CAM = Cd accumulator maize.

Table 4
Blood variables of cattle in Con and CAM on d 40, 70, and 107 of the feeding trials.

Item[1]	d 40		SEM	*P*-value	d 70		SEM	*P*-value	d 107		SEM	*P*-value
	Con	CAM			Con	CAM			Con	CAM		
GLU, mmol/L	5.71	5.16	0.181	0.07	5.00	5.30	0.193	0.55	6.70	6.63	0.461	0.87
BUN, mmol/L	4.75	5.10	0.170	0.44	5.72	5.74	0.148	0.70	3.94	4.75	0.173	0.03
CREA, mmol/L	72.48	73.58	2.251	0.91	79.19	78.24	2.204	0.73	82.08	86.60	2.676	0.53
UA, mmol/L	62.80	51.00	4.070	0.17	35.50	31.33	3.740	0.68	44.00	39.22	4.208	0.68
CHOL, mmol/L	2.83	3.34	0.140	0.24	3.30	3.47	0.148	0.91	2.92	3.51	0.140	0.10
TG, mmol/L	0.37	0.31	0.024	0.06	0.38	0.40	0.019	0.74	0.34	0.38	0.028	0.86
HDL-C, mmol/L	1.92	2.17	0.067	0.22	2.15	2.25	0.073	0.84	2.00	2.11	0.059	0.42
LDL-C, mmol/L	0.84	1.10	0.081	0.16	1.07	1.14	0.079	0.96	0.85	1.33	0.090	< 0.01
SAA, mg/L	0.68	0.46	0.127	0.36	1.16	1.19	0.215	0.99	0.63	0.55	0.116	0.68
C3, μg/mL	108.56	117.43	11.270	0.04	111.32	137.05	12.990	0.02	118.51	128.07	11.764	0.47
C4, μg/mL	347.36	366.92	11.448	0.57	317.52	348.43	12.846	0.34	307.08	387.59	16.761	0.04
IgA, μg/mL	4,806.30	4,662.54	188.616	0.70	5,141.49	4,818.06	158.336	0.44	4,641.93	5,873.78	226.014	< 0.01
IgG, mg/mL	14.13	16.13	0.454	0.03	15.12	16.56	0.467	0.11	14.92	17.56	0.599	0.03
IgM, μg/mL	1,589.19	1,570.89	92.910	0.99	1,628.44	1,897.27	98.820	0.19	1,730.49	1,815.33	57.379	0.45
IL-1β, pg/mL	1,250.87	1,191.68	32.306	0.39	1,167.16	1,230.73	30.376	0.32	1,231.94	1,141.12	33.275	0.20
IL-6, pg/mL	350.80	344.71	10.822	0.81	345.93	353.87	12.388	0.82	372.19	293.48	15.731	< 0.01
IL-22, pg/mL	1,436.52	1,310.04	57.628	0.30	1,351.54	1,327.76	59.931	0.75	1,474.76	1,352.08	52.645	0.64
TNF-α, pg/mL	399.94	377.78	10.651	0.15	377.14	404.52	14.416	0.58	398.16	340.73	13.744	0.06
IFN-γ, pg/mL	1,201.82	1,101.55	82.741	0.73	1,291.49	1,554.73	96.034	0.18	1,336.19	1,400.85	103.555	0.74
IGF-1, ng/mL	293.90	345.46	19.119	0.42	371.38	407.34	18.603	0.16	336.94	338.73	16.571	0.68
LPS, EU/L	1,078.73	1,131.33	34.446	0.57	1,101.94	989.51	33.420	0.04	1,042.22	832.20	51.503	0.03

Con = control; CAM = Cd accumulator maize.
[1] GLU = glucose; BUN = blood urea nitrogen; CREA = creatinine; UA = uric acid; CHOL = cholesterol; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SAA = serum amyloid-A; C3 = complement 3; C4 = complement 4; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-22 = interleukin-22; TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ; IGF-1 = insulin growth factor-1; LPS = lipopolysaccharide.

and C18:0 (16.20% in Con cattle and 15.95% in CAM cattle, Table 7). Other fatty acid proportions were less than 10%. Compared to the Con group, the proportions of C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), and C17:0 ($P = 0.03$) were significantly higher, while the proportions of t11C18:1 ($P = 0.03$) and C20:4 ($P < 0.01$) were significantly lower in the CAM group (Table 7).

Table 5
Meat quality of cattle in Con and CAM.

Item	Con	CAM	SEM	*P*-value
Loin-eye area, cm^2	35.94	37.43	2.139	0.74
pH_30 min	6.48	6.72	0.071	0.10
pH_24 h	5.56	5.56	0.032	0.90
L* (lightness)	31.43	32.85	0.670	0.30
a* (redness)	10.14	10.35	0.693	0.89
b* (yellowness)	2.10	2.64	0.238	0.27
Rate of water loss, %	8.94	8.46	0.613	0.70
Drip loss, %	5.20	5.40	0.400	0.81
Cooked meat percentage, %	36.27	37.45	0.624	0.36
Shear force, N	59.01	64.26	3.599	0.48

Con = control; CAM = Cd accumulator maize.

Table 6
Muscle amino acid composition of cattle in Con and CAM (g/100 g).

Item	Con	CAM	SEM	*P*-value
Essential amino acids (EAA)				
Lys	1.560	1.390	0.0269	< 0.01
Phe	1.126	1.127	0.0138	0.98
Met	0.600	0.580	0.0072	0.18
Thr	0.796	0.802	0.0107	0.78
Val	1.133	1.103	0.0171	0.39
Leu	1.548	1.653	0.0254	0.03
Ile	1.089	1.138	0.0133	0.07
His	0.935	0.922	0.0065	0.35
EAA[1]	8.786	8.713	0.0624	0.58
Non-essential amino acids (NEAA)				
Asp	2.035	2.037	0.0216	0.96
Glu	3.524	3.551	0.0259	0.62
Gly	1.095	1.104	0.0108	0.70
Ala	1.304	1.265	0.0228	0.41
Pro	1.234	1.154	0.0186	0.03
Ser	0.858	0.838	0.0078	0.20
Tyr	0.841	0.835	0.0043	0.53
Cys	0.083	0.079	0.0009	0.01
Arg	1.295	1.233	0.0123	< 0.01
NEAA[2]	12.270	12.096	0.0591	0.15
Total amino acids (TAA)	21.056	20.809	0.1017	0.24
EAA/NEAA, %	71.609	72.053	0.4905	0.66
EAA/TAA, %	41.726	41.864	0.1644	0.69
NEAA/TAA, %	58.274	58.136	0.1644	0.69

Con = control; CAM = Cd accumulator maize.
[1] EAA = Lys + Phe + Met + Thr + Val + Leu + Ile + His.
[2] NEAA = Asp + Glu + Gly + Ala + Pro + Ser + Tyr + Cys + Arg.

Table 7
Muscle fatty acid composition of cattle in Con and CAM (%).

Item	Con	CAM	SEM	*P*-value
SFA[1]	47.70	47.88	0.110	0.43
C8:0	1.58	1.61	0.009	0.10
C10:0	0.46	0.52	0.010	< 0.01
C12:0	0.37	0.42	0.009	< 0.01
C14:0	3.46	3.51	0.025	0.26
C15:0	0.29	0.28	0.004	0.46
C16:0	24.27	24.26	0.126	0.99
C17:0	1.11	1.20	0.020	0.03
C18:0	16.20	15.95	0.091	0.18
C20:0	0.13	0.12	0.003	0.12
MUFA[2]	41.19	41.03	0.118	0.52
C15:1	0.50	0.50	0.006	0.90
C16:1	2.62	2.56	0.017	0.07
C18:1	36.21	36.23	0.126	0.96
Trans-11-C18:1	1.86	1.75	0.027	0.03
PUFA[3]	11.10	11.08	0.053	0.85
C18:2	3.42	3.37	0.050	0.63
C18:3	0.59	0.58	0.006	0.46
Cis-9,trans-11 CLA	0.59	0.60	0.005	0.36
Trans-10,cis-12 CLA	0.21	0.21	0.003	0.79
C20:2	0.18	0.19	0.004	0.22
C20:4	0.77	0.71	0.011	< 0.01
C20:5	3.14	3.19	0.013	0.13
C22:3	0.19	0.20	0.004	0.18
C22:5	1.01	1.03	0.011	0.28
C22:6	0.99	1.00	0.007	0.46

Con = control; CAM = Cd accumulator maize; CLA = conjugated linoleic acid.
[1] SFA (saturated fatty acids) = C8:0 + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0.
[2] MUFA (monounsaturated fatty acids) = C15:1 + C16:1 + C18:1 +t11C18:1.
[3] PUFA (polyunsaturated fatty acids) = C18:2 + C18:3 + C9t11 + t10C12 + C20:2 + C20:4 + C20:5 + C22:3 + C22:5 + C22:6.

4. Discussion

Phytoremediation combined with rational utilization of plant residues could reduce secondary environmental pollution, which would be a useful strategy for Cd phytoremediation in farmland. Anaerobic digestion is an environmentally friendly and cost-effective method that has been used to produce bioenergy from plant residues, while a high concentration of Cd in plant residues is a major factor that reduces the efficiency of anaerobic digestion (Guo et al., 2021). Xu et al. (2017) reported that with 1 mg/L of Cd in the digestion system, the methanogen activity was significantly inhibited. Thus, it is urgent to discover other feasible methods for eliminating Cd phytoremediation plant residues. The present study discovered the possibility of feeding Cd accumulator maize to beef cattle, which can not only provide an efficient usage of these plants but also alleviate the pressure of forage resource shortages in developing countries.

The Cd can be accumulated in kinds of agriculture plant, such as rice (Liu et al., 2022), maize (Yang et al., 2021a), oilseed rape (Zhang et al., 2021) and cotton (Min et al., 2022). In our study, the Cd concentration in the CAM diet was well beyond the Cd restriction in the feed quality standard indicated the ability of maize to perform Cd phytoremediation. Furthermore, though the Cd concentration in the soil that was used to plant the Con group maize is lower than the limitation of GB 15618-2018 (0.12 vs. 0.3 mg/kg) (MEE, 2018), the Cd concentration in the feed used for the Con group was still higher than the Cd restriction (GB 13078-2017) (0.72 vs. 0.5 mg/kg) (SAC, 2017), which confirmed the well ability of maize accumulating of Cd. According to the ADMI and the average BW of the feeding experiment, the Cd consumption per day might be around 0.03 and 0.19 mg/kg of BW in Con and CAM group, respectively. The Cd exposure dose of CAM was (approximately 1/10) lower than the low-dose Cd (1.87 mg/kg of BW) tested in mice (Yang et al., 2015). Yang et al. (2015) reported that with Cd exposure of 1.87 to 7.84 mg/kg of BW in the form of $CdCl_2$ for 10 d, the BW of mice was not affected. Based on the results of Beyrami et al. (2020), mice that received 2 mg/kg of BW Cd intake daily for 30 d showed a significant decrease in BW and feed intake. These results suggested that the effects of Cd on BW and feed intake were both affected by dose and exposure time. At the present Cd exposure dose at a duration of 107 d, the BW of CAM cattle was not affected and the ADMI was decreased. The reduction in feed intake could be associated with the effect of Cd on appetite (Heydarnejad et al., 2013). The decreased F:G ratio suggested higher feed efficiency in CAM cattle. In ruminants, as much as 70% of the daily energy requirement is provided by short-chain fatty acids generated by rumen microbial fermentation (Yeoman and White, 2014). It was reported that the gut microbial community of mice was affected by Cd exposure, including increased abundances of *Bacteroidales S24-7*, Coriobacteriaceae, *Lactobacillus*, *Ruminococcaceae_UCG-010,* and *Clostridium* and decreased abundances of Lachnospiraceae and *Prevotella* (Yang et al., 2021b; Li et al., 2020; Ba et al., 2017). These are common bacteria colonized in the rumen that play important roles in carbohydrate metabolism. Furthermore, the gut content metabolites of rats changed by Cd exposure were enriched in the

metabolic pathways of starch and sucrose, propanoate, butanoate, pyruvate, and so on (Yang et al., 2021b). These results suggested that Cd might promote fiber fermentation and increase energy substrates for cattle, thus, leading to better feed efficiency. However, the changes in the rumen bacterial community and bacterial fermentation need further investigation for confirmation.

Since the CAM diet seemed to have little negative effect on the growth performance of cattle, we further investigated the metabolism and health risk suffered by CAM cattle and evaluated the quality and safety of their products. Among the serum physiological variables, BUN represents the nitrogen supply from the urea cycle to the rumen for microbial growth and microbial protein biosynthesis. A higher BUN concentration in CAM cattle indicated better efficiency in utilizing nitrogen resources in beef cattle. LDL-C is the main carrier of blood CHOL (Ntaios and Milionis, 2019), and blood CHOL is closely associated with liver metabolism (Van Soest, 2018). The increased serum LDL-C and CHOL levels suggested a CHOL redistribution tendency from the liver into the serum induced by the CAM diet (Wang et al., 2018), which would further lead to a higher carcass fat content in CAM cattle (Wheeler et al., 1987). On a note, the serum CHOL level was still in the normal range.

Cd potentially triggers the immune system and induces acute/chronic inflammatory responses locally/systemically (Ghosh and N, 2018; Guo et al., 2020; Ge et al., 2021); thus, serum inflammatory and immunological variables were measured. Blood immunoglobulin has the ability to act as an antibody and plays a very important role in preventing infections (Alkan Ozdemir et al., 2016). C3 and C4 play key roles in the complement system (Reis et al., 2019), which is critically involved in the pathogenesis of inflammatory and immune complex diseases (Ricklin et al., 2016). The increased serum IgA, IgG, C3 and C4 in CAM cattle suggested an increased ability to protect cattle from the inflammation induced by Cd exposure. IL-6 is a pleiotropic cytokine that targets B cells, T cells, and hepatocytes and works as the primary regulator of the acute-phase response (Castell et al., 1989). Consistent with the suppressed IL-6 production in the monocytic cell line by low Cd exposure (0.06 to 0.1 mmol/L) (Funkhouser et al., 1994), we observed a decreased concentration of serum IL-6 in CAM cattle, which suggested a risk in the host innate immune response. LPS is a proinflammatory factor produced by Gram-negative bacteria (Hamann et al., 1998). It was reported that serum LPS was increased in 4 mg/kg Cd recipient rats (Yang et al., 2021b). In contrast, the serum LPS level was decreased in the cattle fed the CAM diet, but whether this was associated with the changes in the gut microbial community requires further investigation.

Cd accumulation in the visceral organs of CAM cattle observed in this study indicated that these visceral organs could not be used for human/animal food/feed (GB 2762-2022; GB/T 31216-2014; GB/T 31217-2014) (NHC, 2022, SAC, 2014a,b). Among them, the kidney is the main target of Cd toxicity, and Cd in the state of ions or protein complexes can be filtered or accumulated in renal tubular cells, eventually causing cell damage (Prozialeck and Edwards, 2012). In Cd-poisoned mice, the kidney showed swelling and necrosis of renal tubular epithelial cells and irregular glomerular structure (Chen et al., 2018). Under low exposure to Cd, we observed a change in the structure of the kidney in CAM cattle, while its function was not affected, as reflected by the normal concentration of BUN, an index for the clinical evaluation of renal function.

The Cd residue in the muscle of CAM cattle was 107 μg/kg, which was 7 μg/kg beyond the restriction for human food (GB 2762-2022). A lower dose of CAM could further be investigated to restrict the muscle Cd residue within 100 μg/kg. Along with the increased Cd residue in the muscle of CAM cattle, the compositions of amino acids and fatty acids were changed, which are key factors for evaluating the nutritional value and flavor of meat (Cabrera and Saadoun, 2014; Chai et al., 2018; Khan et al., 2015). The Maillard reaction between Cys and sugar during cooking is crucial to the formation of meaty aroma (Hou et al., 2017). Pro imparts sweetness to the meat (Ramalingam et al., 2019), However, Leu imparts bitterness to meat (Ramalingam et al., 2019) and was reported to be negatively correlated with beef volatile flavor compounds (Huang et al., 2022). Compared with the Con group, the content of Cys and Pro in the CAM group decreased while Leu increased, which suggested that the CAM diet negatively affect the flavor of meat. Conjugated linoleic acid (CLA) has positive effects on anti-tumor, anti-obesity, and anti-cardiovascular diseases (Zeng et al., 2020; DeClercq et al., 2012). In mammalian adipose tissue, t11C18:1 is an important precursor for the synthesis of CLA (Wang et al., 2020). Arachidonic acid (C20:4) is an essential fatty acid that regulates complex cardiovascular functions (Zhou et al., 2021). Higher concentration of C20:4 can effectively improve chicken meat flavor (Takahashi, 2018). Lauric (C12:0) has been reported to cause the risk of atherosclerosis and hyperlipidemia (Ulbricht and Southgate, 1991). The t11C18:1 and C20:4 were decreased, while the C12:0 was increased in the muscle of CAM cattle, which suggested a decrease in meat nutritional value and flavor.

5. Conclusion

At the current feeding level, phytoremediation maize did not affect the BW but decreased the ADMI and increased the feed efficiency of beef cattle. The increased Cd level in the diet (the concentration of Cd in feedstuff used to feed beef cattle exceeded the Hygienical Standard for Feeds) increased the health risk in those cattle, as reflected by increased serum LDL-C, CHOL, IgA, IgG, C3, and C4 and decreased serum IL-6, as well as Cd accumulation in visceral organs and structural changes in the kidney. The meat of CAM cattle was not suitable for human consumption, since the Cd residue was too high, and the meat nutrition and flavor were also affected by changing the composition of amino acids and fatty acids. Therefore, further research must be performed to determine whether a lower proper dose of phytoremediation maize and an appropriate feeding period may be possible to ensure safe feed for beef cattle and safe meat products (< residue limit) for humans. Furthermore, whether long-term ingestion of Cd contaminated meat at low levels (< residue limit) cause health risk in human body should be considered.

Author contributions

Zebang Xu: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Bin Yang**: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Kangle Yi**, **Tianrong Chen**, **Xinxin Xu**, **Ao Sun**, **Haobang Li**, **Jianbo Li**, **Fang He** and **Cheng Huan**: Investigation. **Yang Luo**: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Jiakun Wang**: Conceptualization, Supervision, Writing – review & editing. We declare that all authors have read and agree with the manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

The research was supported by the Department of Agriculture and Rural Affairs of Hunan Province, the Construction of Modern Agricultural Industrial Technology System in Hunan Province (Hunan Financial Agriculture Guide 2019[97]).

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