Chromium concentrations in ruminant feed ingredients

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ABSTRACT

Chromium (Cr), in the form of Cr propionate, has been permitted for supplementation to cattle diets in the United States at levels up to 0.50 mg of Cr/kg of DM since 2009. Little is known regarding Cr concentrations naturally present in practical feed ingredients. The present study was conducted to determine Cr concentrations in feed ingredients commonly fed to ruminants. Feed ingredients were collected from dairy farms, feed mills, grain bins, and university research farms. Mean Cr concentrations in whole cereal grains ranged from 0.025 mg/kg of DM for oats to 0.041 mg/kg of DM for wheat. Grinding whole samples of corn, soybeans, and wheat through a stainless steel Wiley mill screen greatly increased analyzed Cr concentrations. Harvested forages had greater Cr concentrations than concentrates, and alfalfa hay or haylage had greater Cr concentrations than grass hay or corn silage. Chromium in alfalfa hay or haylage ($n = 13$) averaged 0.522 mg/kg of DM, with a range of 0.199 to 0.889 mg/kg of DM. Corn silage ($n = 21$) averaged 0.220 mg of Cr/kg of DM with a range of 0.105 to 0.441 mg of Cr/kg of DM. By-product feeds ranged from 0.040 mg of Cr/kg of DM for cottonseed hulls to 1.222 mg of Cr/kg of DM for beet pulp. Of the feed ingredients analyzed, feed grade phosphate sources had the greatest Cr concentration (135.0 mg/kg). Most ruminant feedstuffs and feed ingredients had less than 0.50 mg of Cr/kg of DM. Much of the analyzed total Cr in feed ingredients appears to be due to Cr contamination from soil or metal contact during harvesting, processing, or both.

Key words: chromium, cattle, feed ingredients, ruminants

INTRODUCTION

Chromium (Cr) potentiates the action of insulin in insulin-sensitive tissues, and based on research in hu-

mans, the Institute of Medicine established an adequate intake of Cr for humans (National Academies, 2001). Chromium concentrations in foods and diets consumed by humans have also been reported (Anderson et al., 1992).

Before the early 1990s, practical diets fed to cattle were generally assumed to provide adequate Cr to meet dietary requirements. However, studies conducted in the early 1990s indicated that Cr supplementation could improve performance and reduce morbidity in stressed calves (Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993). Subsequent research demonstrated that Cr supplementation during late gestation and early lactation increased milk production in dairy cows (Yang et al., 1996; Hayirli et al., 2001).

The US Food and Drug Administration Center for Veterinary Medicine issued a regulatory discretion letter in 2009, which permitted the use of Cr propionate (**Cr Prop**) as a source of Cr for supplementation to cattle diets at levels up to 0.5 mg of Cr/kg of DM. Chromium supplementation from Cr Prop was found to enhance insulin sensitivity in growing cattle (Spears et al., 2012). Recent studies have indicated that Cr Prop supplementation may enhance performance and health of stressed calves (Bernhard et al., 2012) and increase milk production in dairy cows (Vargas-Rodriguez et al., 2014; Rockwell and Allen, 2016).

Little is known regarding Cr concentrations naturally present in practical feedstuffs. Chromium analysis of feed ingredients and total diets is challenging because of the low concentration of Cr normally present and problems with Cr contamination of samples during collection, processing, and laboratory preparation of samples for analysis (NRC, 2005). Mean Cr concentrations in homegrown feedstuffs in Wisconsin ranged from 0.33 mg/kg of DM for corn grain to 0.91 mg/kg of DM for alfalfa haylage (Li et al., 2005). Average Cr concentrations in unground wheat samples from different areas in Australia ranged from 0.013 to 0.041 mg/kg, and Cr concentrations in oats and barley were 0.020 and 0.024 mg/kg, respectively (Jones and Buckley, 1977). The objectives of the present study were to determine Cr concentrations in feed ingredients commonly fed to ruminants and to determine the extent of variation that

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Table 1. Summary of feed ingredients analyzed

Feedstuff or ingredient	Form ¹	Further processing	Location ²
Alfalfa hay or haylage	As fed	Wiley mill	CA, IA, ID, MI, NC, NM, WI, MO
Beet pulp	Pelleted/shreds	Wiley mill	MO, CA, KS
Calcium carbonate	Granular	None	NC, IA
Corn	Whole	None	IA, IL, IN, KS, MI, MN, OH, NE
Corn	Cracked, flaked, or rolled	Wiley mill	IN, KS, IA, MN, OH, IL, ID, MI, KY, NC, CA, NM, NY
Corn distillers dried grains with solubles (DDGS)	Fine or coarse particle	None	IA, NC, SD, NY
Corn silage	As fed	Wiley mill	CA, ID, MI, NC, NH, NY, VT, WI
Corn steep liquor	Liquid	None	IA, Canada
Cottonseed hulls	As fed	None	KY, MI
Cottonseed, whole	Whole	Wiley mill	KY, NC
Grass hay	As fed	Wiley mill	NY, NC, MI
Grasses	Clippings	Wiley mill	NC
Molasses, cane	Liquid	None	LA, FL
Oats	Whole	None	IA, MI
Phosphate supplements	Granular	None	NC, FL, South Africa
Soybeans	Whole	None	IA, IN, KS, NE
Soybean hulls	Loose	None	KY, NC
Soybean hulls	Pelleted	Wiley mill	KY, MI, NC, MO
Soybean meal	Granular	None	KS, MI, MN, MO, NC
Wheat	Whole	None	KS, TX
Wheat middlings	Flakes	None	MN, NE, IA, IN, MI
White clover	Clippings	Wiley mill	NC
Yeast, cultures	Powder	None	KS

¹Form in which feed was received.

²Location (state or country) from where feedstuff or ingredient was received. CA = California, IA = Iowa, ID = Idaho, MI = Michigan, NC = North Carolina, NM = New Mexico, WI = Wisconsin, MO = Missouri, KS = Kansas, IL = Illinois, IN = Indiana, MN = Minnesota, OH = Ohio, NE = Nebraska, KY = Kentucky, NY = New York, SD = South Dakota, NH = New Hampshire, VT = Vermont, LA = Louisiana, FL = Florida, TX = Texas.

exists within several feed ingredients derived from different geographic areas throughout the United States.

MATERIALS AND METHODS

Feed ingredients were collected from dairy farms, feed mills, grain bins, and research farms at North Carolina State University (NCSU) and Michigan State University. The number of samples for the various feed ingredients analyzed ranged from $n = 2$ to $n = 21$. A summary of the feed ingredients analyzed along with the form received, whether feed ingredients were ground in the laboratory, and the states where feed ingredients were derived is shown in Table 1. All samples were dried in a forced-air oven at 55°C, and results are expressed on a DM basis.

Forages

Samples of silage, hay, and haylage were obtained from dairy farms, NCSU, and Michigan State University. Hand-clipped samples of KY 31 tall fescue, Max Q tall fescue, orchardgrass, and white clover were collected at 2 vegetative stages (April 14 and May 14) from pastures at NCSU. Forage samples were clipped

approximately 5 cm above the ground. Samples were taken from multiple sites within a single pasture of each forage. Following drying, all forage samples were ground through a 1-mm stainless steel screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA).

Concentrates

Unprocessed samples of corn, wheat, oats, and soybeans were collected from feed mills or on-farm grain bins and prepared for Cr analysis without grinding to minimize Cr contamination. Samples of soybean meal and ground corn obtained from feed mills were also analyzed for Cr without further processing. Cracked and flaked corn samples were ground in a Wiley mill before Cr analysis.

By-Product Feedstuffs and Miscellaneous Feed Ingredients

By-product feed ingredients were obtained from feed mills or from farms and were not processed further before Cr analysis, unless they were received in a pelleted form. All liquid feed ingredients were obtained from Quality Liquid Feeds (Dodgeville, WI). Liquid

feed ingredients were wet ashed for Cr analysis without drying.

Cr Contamination from Grinding

To determine the effect of grinding grain samples through a laboratory Wiley mill on analyzed Cr concentrations, samples of whole corn ($n = 8$), soybeans ($n = 7$), and wheat ($n = 5$) were prepared for Cr analysis without further processing. A portion of these same grain samples were then ground through a 1-mm stainless steel screen before preparation for Cr analysis.

Subsampling

The amount of each feed ingredient sample collected ranged from 100 g to 1 kg. All ground samples were initially mixed by rotation in their storage bags. All contents were then poured out onto a piece of brown kraft paper large enough to accommodate the entire sample. The sample was further mixed by turning over the consecutive corners of the brown kraft paper multiple times, which in turn mixed the bottom portion with the top portion of the sample. The ground sample was then divided into 4 equal quadrants. One of the quadrants was then subsampled at a minimum of 5 locations until the ashing/digestion weight was obtained.

Unground samples of grains were dried in the oven and stored in bags in the same fashion as the ground samples. Storage bags were thoroughly mixed by rotation, and the sample particles were removed with plastic tweezers from several locations in the bag until the ashing weight was obtained.

Cr Analysis

Feed ingredient samples were prepared for Cr analysis by wet ashing with trace mineral grade nitric acid (Trace Metal grade, Fisher Scientific, Raleigh, NC). Briefly, for all samples except mineral supplements (calcium carbonate and phosphate sources), an amount weighing between 0.5 and 1.0 g was predigested at room temperature for 18 to 24 h with 10 mL of nitric acid in a 50-mL polypropylene tube. Predigested samples were ashed further using the CEM Hi-Thru Put Method with a Mars5 Microwave (CEM, Matthews, NC). Once microwaving was complete, all acid-digested samples were brought up to a final volume of 25 mL with deionized H₂O to allow minimal dilution for Cr analysis. No particles were present following wet ashing, indicating that acid hydrolysis was complete even for samples that were not ground. For mineral supplements, approximately 0.2 g was exposed to nitric acid for 18 to 24 h at

room temperature and then brought up to a final volume with deionized H₂O. Calcium carbonate and phosphate sources were analyzed for Cr using flame atomic absorption spectrophotometry (model 6701, Shimadzu, Kyoto, Japan) because of the high concentration of Cr present in these samples. Chromium in all other feed ingredients was measured by electrothermal atomic absorption spectrophotometry (GFA-6500, Shimadzu) as described by Lloyd et al. (2010). The method of standard addition was used to remove matrix effects. Each sample plus the standard was run in duplicate. Bovine muscle obtained from the National Institute of Standards and Technology (Washington, DC) and certified to contain 71 ± 38 ng of Cr/g was ashed and analyzed in the same manner as feed ingredient samples. The reference standard was analyzed to contain 81.5 ± 1.5 ng Cr/g.

Statistical Analysis

Means, SD, and ranges of Cr concentrations are presented for each feed ingredient. Whole versus ground samples of grain were analyzed by grain type as a randomized block design by ANOVA using the MIXED procedure of SAS (SAS Institute, 2002). The model included block (sample) and treatment.

RESULTS AND DISCUSSION

Chromium concentrations in concentrate feeds are presented in Table 2. Mean Cr concentrations in whole cereal grains were low, ranging from 0.025 mg/kg of DM for oats to 0.041 mg/kg DM for wheat. Analyzed Cr concentrations obtained in the present study for oats and wheat were in line with those reported previously (Welch and Cary, 1975; Jones and Buckley, 1977; Cary and Kubota, 1990). Chromium levels in wheat samples collected from different areas in Australia ranged from 0.009 to 0.090 mg/kg DM (Jones and Buckley, 1977). Wheat samples from different US states analyzed from 0.003 to 0.038 mg of Cr/kg of DM (Welch and Cary, 1975). Samples of oats collected in Australia averaged 0.020 mg of Cr/kg of DM (Jones and Buckley, 1977). Chromium concentrations in whole corn samples in our study were lower than those reported for corn grain samples collected from dairy farms in Wisconsin (Li et al., 2005). This finding could be due to differences in the amount of Cr contamination during sampling, preparation of samples for analysis, or both or differences in analytical procedures used to measure Cr. Corn samples from the Wisconsin study were ground through a 1-mm plastic screen before ashing, but samples in the present study were not processed before ashing.

Table 2. Chromium concentrations (mg/kg of DM) in concentrate and forage feed ingredients

Feedstuff	No. of samples	Mean	SD	Range
Concentrate				
Corn, whole	16	0.026	0.015	0.008–0.054
Corn, processed	16	0.049	0.031	0.014–0.114
Cottonseed	2	0.094	0.086	0.033–0.155
Oats, whole	3	0.025	0.008	0.021–0.034
Soybeans, whole	7	0.069	0.035	0.034–0.122
Soybean, meal	6	0.208	0.050	0.154–0.286
Wheat, whole	6	0.041	0.014	0.029–0.062
Forage				
Alfalfa hay or haylage	13	0.522	0.220	0.199–0.889
Corn silage	21	0.220	0.087	0.105–0.441
Grass hay	5	0.155	0.093	0.098–0.320
Clipped forage				
Orchardgrass	2	0.101	0.010	0.094–0.108
Tall fescue	4	0.179	0.074	0.112–0.248
White clover	2	0.358	0.046	0.325–0.390

Processed (cracked or flaked) corn samples received and then ground through a stainless steel screen had Cr concentrations almost twice as high as those in whole corn samples (Table 2). Whole cottonseed and soybeans had slightly greater Cr concentrations than the cereal grains. Soybean meal samples contained the greatest Cr concentrations of the concentrate feeds evaluated in this study.

Harvested forages contained greater Cr concentrations than concentrates (Table 2). Alfalfa hay or haylage (0.522 mg of Cr/kg of DM) contained greater mean Cr concentrations than corn silage (0.222 mg/kg of DM) or grass hay (0.155 mg/kg of DM). Li et al. (2005) also reported greater Cr concentrations in alfalfa hay and haylage compared with corn silage. The greater Cr levels in forages than in concentrates is not surprising. Previous research indicated that the grain portion of wheat and oats is much lower in Cr than the plant leaf and stem (Cary and Kubota, 1990). Chromium concentrations in clipped samples of cool season grasses

ranged from 0.094 to 0.248 mg of Cr/kg of DM (Table 2). White clover contained greater Cr concentrations than grasses. Subterranean clover samples collected in Australia also contained greater (0.76 vs. 0.44 mg/kg of DM) Cr concentrations than ryegrass samples (Jones and Buckley, 1977).

Chromium concentrations in by-product and miscellaneous feed ingredients are shown in Table 3. Beet pulp (mean = 1.222 mg/kg of DM) and corn steep liquid (1.053 mg/kg of DM) contained the greatest Cr concentrations of the by-product feed ingredients analyzed. The greater Cr concentrations in beet pulp may be due to greater soil contamination because beets grow underground. With the exception of beet pulp and corn steep liquid, other by-product feed ingredients averaged less than 0.60 mg of Cr/kg of DM. Pelleted samples of soybean hulls had greater Cr concentrations than samples of soybean hulls received in a loose form. The greater Cr concentration in pelleted samples may be due to Cr contamination occurring during pelleting

Table 3. Chromium concentrations (mg/kg of DM) in by-product and miscellaneous feed ingredients

Feedstuff	No. of samples	Mean	SD	Range
Beet pulp	3	1.222	0.386	0.776–1.451
Calcium carbonate	3	6.79	1.08	5.63–7.78
Corn steep liquor	2	1.053	0.426	0.752–1.355
Cottonseed hulls	2	0.040	0.013	0.031–0.050
DDGS ¹ loose	12	0.160	0.056	0.084–0.238
Molasses, cane	2	0.557	0.049	0.522–0.591
Phosphate sources	5	135.0	19.8	112.0–163.0
Soybean hulls, loose	3	0.262	0.073	0.191–0.336
Soybean hulls, pelleted	4	0.550	0.175	0.309–0.705
Wheat middlings	9	0.084	0.031	0.044–0.132
Yeast, cultures	2	0.600	0.091	0.536–0.664

¹DDGS = distillers dried grains with solubles.

or during grinding of pelleted samples in the laboratory. Pelleted samples were ground through a stainless steel screen, but loose samples were not. By-product feed ingredients generally contained greater Cr concentrations than the whole grains analyzed.

Calcium carbonate sources averaged 6.79 mg of Cr/kg, and phosphate sources averaged 135.0 mg of Cr/kg (Table 3). Phosphate sources analyzed included monocalcium phosphate, dicalcium phosphate, and defluorinated phosphate. Chromium would be expected to be present as a contaminant in mineral ingredients, and studies (Sullivan et al., 1994; Lima et al., 1995) have indicated that most feed-grade phosphate sources are high in Cr. Chromium concentrations in 9 samples of mono-dicalcium phosphate produced in the United States averaged 203 mg/kg, with a range of 65 to 538 mg/kg (Sullivan et al., 1994).

Most complete diets and feedstuffs are typically ground through a Wiley mill screen before laboratory analysis. Table 4 shows a comparison of analyzed Cr concentrations in whole samples of corn, soybeans, and wheat versus these same samples following grinding through a stainless steel Wiley mill screen. Analyzed Cr concentrations in all grains were significantly increased by grinding. Grinding increased analyzed Cr values in corn by 150%, wheat by 113%, and soybeans by 87%.

Accurately measuring naturally occurring Cr in feed ingredients is difficult because of Cr contamination that can occur during harvesting, processing, and collection of samples as well as laboratory analysis of Cr. Chromium is ubiquitous in nature, occurring in soils, plants, water, and air. It is naturally present primarily in the trivalent form as ferrochromite (FeCr_2O_4) ores (Katz and Salem, 1994). A large portion of Cr ores are processed for use in the production of stainless steel and other alloys. Chromium is also used as a component of chrome and in chrome plating, leather tanning, and the manufacturing of pigments and wood preservatives. The present study clearly demonstrates that the grinding of whole grain samples through a stainless steel screen significantly increased analyzed Cr concentrations. During feed harvesting and processing, it should be recognized that numerous opportunities exist for Cr contamination from metal contact.

Soil contamination can also contribute to the total Cr concentration in feed ingredients and feeds. Cary and Kubota (1990) estimated soil contamination of plant tissues using titanium as a marker of the degree of soil contamination. They found that soil contamination was an important factor contributing to the total Cr concentration in most plant tissue samples analyzed. The amount of soil contamination in plant samples from this study was likely less than what occurs during

Table 4. Effect of grinding through a stainless steel Wiley mill screen on chromium concentrations of grains

Grain	No. of samples	Cr, mg/kg of DM		P-value
		Whole	Ground	
Corn	8	0.020	0.050	0.01
Soybeans	7	0.077	0.144	0.02
Wheat	5	0.045	0.096	0.03

production scale harvesting of hay and silage. Rafferty et al. (1994) found that soil contamination averaged less than 2% when hay and grass silage samples were obtained fresh before harvest. However, soil contamination rates as high as 8% were observed in some samples collected after harvesting.

Analytical determination of Cr also represents another source of variability and error in accurately reporting Cr concentrations in feed ingredients because of the low Cr concentrations present and Cr contamination from reagents used in preparation of samples for analysis. Many Cr supplementation studies in the past have not included an analysis of experimental diets for Cr (Hayirli et al., 2001; Van Bibber-Krueger et al., 2016) or have used flame atomic absorption spectroscopy to measure Cr in diets (Edenburn et al., 2016; Kneeskern et al., 2016). Chromic oxide has frequently been used as a nondigestible marker for digestibility studies. Flame atomic absorption can detect the high concentrations of Cr found in feed and fecal samples from animals given chromic oxide as a nondigestible marker. However, flame atomic absorption spectrometry is not sensitive enough to detect the low concentrations of Cr found in most feedstuffs (NRC, 2005). In the present study we attempted to minimize Cr contamination of samples. We could not control Cr contamination that occurred during harvesting of forages or grains, processing of feed ingredients, or grinding of certain feed ingredients through a stainless steel screen in the laboratory. However, the graphite furnace atomic absorption procedure used in the present study is highly sensitive for measuring low concentrations of Cr and has a detection limit of 0.005 μg of Cr/L when an appropriate background correction method is used (NRC, 2005). Nitric acid of the highest purity was used in preparing samples for analysis to avoid Cr contamination. A sample from the National Institute of Standards and Technology with a certified Cr concentration was also used as a reference standard. Furthermore, our Cr analytical procedure has been validated according to CLSI guidelines for a variety of matrices and was reviewed and accepted by the FDA as part of a food additive petition (21CFR573.304).

Consistent with previous research with human plant foods (Welch and Cary, 1975; Cary and Kubota, 1990), the present study clearly indicates that Cr uptake by most plants consumed by cattle is low. Chromium is found in soils primarily in insoluble forms (Zayed and Terry, 2003). Soluble forms of Cr added to soil are rapidly converted to insoluble forms (Cary et al., 1977a). Chromium taken up from soils by plants is also poorly translocated from the root to other parts of the plant (Cary et al., 1977b; Lahouti and Peterson, 1979). However, plants appear to vary with regard to Cr translocation from their roots (Cary et al., 1977a; Johnson and Weaver, 1986). For example, kale accumulated more Cr than the edible tissues of wheat and legumes (bush beans and soybeans; Johnson and Weaver, 1986). The present study suggests that legumes are higher in Cr than grasses. Alfalfa hay and haylage contained greater Cr concentrations than grass hay, and clipped samples of white clover had greater Cr concentrations than clipped samples of grass. Jones and Buckley (1977) reported that subterranean clover contained greater Cr concentrations than ryegrass.

Studies in humans (Anderson and Kozlovsky, 1985) and rats (Johnson and Weaver, 1986) have indicated that the bioavailability of Cr naturally present in foods is low. Chromium absorption from self-selected diets of humans ranged from 0.5 to 2.0% (Anderson and Kozlovsky, 1985). Retention of ^{51}Cr from kale, wheat, and eggs intrinsically labeled with ^{51}Cr ranged from 1.1 to 1.7% of dose by d 9 after dosing (Johnson and Weaver, 1986). Bioavailability of Cr from ruminant feedstuffs and feed ingredients has received little attention. Animal feed ingredients would consist of a combination of Cr naturally occurring in feeds and Cr resulting as a contaminant from soil, metal contact, or both. Chromium in soil and from metal contamination during harvesting or processing is probably unavailable or at least of extremely low bioavailability to ruminants. Acid conditions occurring during the fermentation of silage or haylage may increase the solubility and thus the bioavailability of soil Cr. Water solubility of iron from soil was increased following fermentation of corn silage (Hansen and Spears, 2009). In alfalfa (Blincoe, 1974) and beans (Huffman and Allaway, 1973) an anionic, low-molecular-weight water-soluble Cr complex has been characterized. Starich and Blincoe (1983) reported that rats absorbed approximately 30% of Cr from ^{51}Cr -labeled alfalfa. In contrast, rats fed ^{51}Cr -labeled bean leaves retained less than 0.5% of the dose after 48 h (Huffman and Allaway, 1973). Chromium propionate has been shown to provide bioavailable Cr, based on its ability to enhance insulin sensitivity in cattle fed a basal diet containing 0.20 mg of Cr/kg of DM (Spears et al., 2012).

CONCLUSIONS

Chromium concentrations in most ruminant feedstuffs and feed ingredients are low. Mean Cr concentrations in unprocessed cereal grains are less than 0.05 mg/kg of DM. Harvested forages contained greater Cr concentrations than grains, with mean Cr concentrations ranging from 0.155 mg/kg for grass hay to 0.522 mg/kg for alfalfa hay or haylage. By-product feed ingredients ranged from 0.040 mg/kg for cottonseed hulls to 1.222 mg/kg for beet pulp. Grinding whole grain samples through a stainless steel Wiley mill screen greatly increased analyzed Cr concentrations. Much of the analyzed total Cr in feed ingredients appears to be due to Cr contamination from soil or metal contact during harvesting, processing, or both. This study indicates that the permitted concentration (0.50 mg of Cr/kg of DM) of Cr Prop that can be supplemented to cattle diets is significant with regard to total dietary Cr intake.

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