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Ecosystem Abstract <sup>3</sup>Desert Agriculture and Program, Kuwait Institute for Scientific Research (KISR), P.O. Box. 24885, Safat 13109 Kuwait, Kuwait.

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# Assessment of heavy metals in feed and beef in Bangladesh: A safety

issues

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The study was aimed to assess the heavy metal status in feed and beef produced in Bangladesh. In the study, the quantitative presence/absence of heavy metals in livestock feed ingredients, beef feed and their residues in beef of four district of Bangladesh were investigated. Feeds and meats were analyzed by Atomic Absorption Spectrophotometer (AAS) for heavy metals such as lead (Pb), Chromium (Cr), Cadmium (Cd), Zinc (Zn) and Copper (Cu). Concentration of Zn, Cu, Cr, and Pb in feed ingredients ranged from 0.124 to 1.209 mg/kg, 0 to 0.215 mg/kg, 0.095 to 0.286 mg/kg and 0.164 to 0.301 mg/kg, respectively. In all feed ingredients mean concentration of Zn was higher than other heavy metals. Concentration of Zn, Cu, Cr, and Pb in commercial beef cattle feed ranged from 0.313 to 1.261 mg/kg, 0.004 to 0.140 mg/kg, 0.223 to 0.459 mg/kg and 0.218 to 0.361 mg/kg, respectively. Pb concentrations were  $0.084 \pm 0.046$ ,  $0.364 \pm 0.202$ ,  $0.263 \pm 0.056$ ,  $0.278 \pm 0.089$ ,  $0.094 \pm 0.0466$  mg/hg in heart, kidney, liver, lung and muscle, respectively. Cd concentrations were  $0.003 \pm 0.001$ ,  $2.339 \pm 0.712$ ,  $0.299 \pm 0.221$ ,  $0.024 \pm 0.005$ ,  $0.014 \pm 0.009$ mg/hg in heart, kidney, liver, lung and muscle, respectively. Cr concentrations were  $0.555 \pm 0.451$ ,  $0.164 \pm 0.039$ ,  $0.199 \pm 0.091$ ,  $0.512 \pm 0.018$ ,  $0.232 \pm 0.023$  mg/hg in heart, kidney, liver, lung and muscle, respectively. Zn concentrations were  $6.059 \pm 3.362$ ,  $9.984 \pm 1.358$ ,  $14.776 \pm 0.816$ , 1.567 $\pm$  2.625, 15.883  $\pm$  0.438 mg/hg in heart, kidney, liver, lung and muscle, respectively. Cu concentrations were  $6.059 \pm 3.362$ ,  $9.984 \pm 1.358$ ,  $14.776 \pm 0.816$ ,  $11.567 \pm 2.625$ ,  $15.883 \pm 0.438$ mg/hg in heart, kidney, liver, lung and muscle, respectively which was higher in different organs of beef cattle. The results indicate that the heavy metal residues in feed and feed ingredients were below the maximum residue level (MRL) values, whereas Cu in different organs was slightly higher. Heavy metal residues in feed were in lower concentrations except cadmium, yet below the maximum residue level (MRL). Contaminants existed at different levels in cattle feed and beef of Bangladesh.

# Introduction

Livestock is one of the most important sub-sectors of agriculture which plays a vital role in promoting national economy of Bangladesh (Kamal et al., 2019). Feed and food safety are the main issues in public health in Bangladesh (Hashem et al., 2020). Poultry, goats/sheep and cattle produced 40%, 12% and 48%, respectively. The safety of livestock products directly related to feeds (Kamal et al., 2016 and 2020). Food safety hazards associated with animal feed can be biological, chemical or physical (Rahman et al., 2020). Each hazard is associated with particular sources and routes of contamination and exposure. Animal feeds and forages contain a wide range of contaminants and toxins arising from anthropogenic and natural sources (Baset et al., 2003; Hossain et al., 2016; Huq, 1996; Rahman et al., 2008, 1998 and 1999; Sarkar et al., 2008). In this report, the distribution of heavy metals in feed ingredients, complete feeds and beef meat and organs is presented. Heavy metals are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water (Tchounwou et al., 2012) and their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment. Heavy metals including copper (Cu), cadmium (Cd), chromium (Cr), and lead (Pb) are potential bio-accumulative toxicants that may cause severe health problems even at low concentrations (Ali et al., 2013). Several heavy metals are essential elements and added to animal feed to balance the micro minerals (Dai et al., 2016). One of the major aims of the study was to gain insight into the contamination with zinc, copper, chromium, lead and cadmium in feed ingredients, compound cattle feed and beef. The results can be used to define priorities for national monitoring plans. The increasing demand of food safety has accelerated research regarding the risk associated with consuming food contaminated by these hazardous elements.

# **Methods and Materials**

# **Collection of Samples**

Sampling was done by taking three (3) individual samples and bulking these together to provide one sample of 500 g in an air tied zipper bag for analysis at different places of Bangladesh from farmers engaged with beef cattle fattening. Commonly used raw materials used for beef cattle ration and available commercial feeds, namely ACI, Care Feed, Gain feed, Teer, Saudia, United

Feed, Provita, Index and IFAEL were sampled. Meat sample were collected during Eid-Ul-Adha festival.

#### Sample pretreatment

The samples were oven-dried at 105°C to remove all moisture and thereby the moisture contents of the samples were determined. The samples were oven-dried until the difference between two readings of weight was found negligible.

#### Sample preparation method for heavy metal analysis

The samples were prepared by using  $HNO_3$  - $HCIO_4$  digestion (Kebbekus and Mitra, 1998).  $HNO_3$  -  $HCIO_4$  = 2:1 digestion was preferred over the more common  $HNO_3$  extraction for the determination of heavy metals.

#### Sample analysis

The sample solutions were analyzed for heavy metal contents using graphite furnace atomic absorption spectrophotometer (Model No: SHIMADZU AA-7000). Measurements were made using the hollow cathode lamps for Pb, Cd, Cr, Zn and Cu at the proper wave length and other AAS conditions were employed in the determinations.

### **Statistical analysis**

The data that were obtained from the experiments were statistically analyzed to find the mean and standard deviation among the various samples in triplicate. Data were analyzed using the software, IBM SPSS Statistics, version 20 at the 0.05 level.

#### **Results and discussion**

#### **Concentration of heavy metals in feed ingredients**

The ingredients analyzed were: sesame oil cake, wheat bran, gram (chick pea) bran/hulls, corn grain, mustard oil cake, rice polish (de-oiled), rape seed meals, soybean meals, lentil hulls and cotton by-products. The analytical results have been presented as follows (Table 1):

**Zinc (Zn):** Concentrations of Zn were found within the range from 0.124 ppm to 1.209 ppm (Table 1). Mean concentration of Zn was highest in de-oiled rice polish  $(1.204 \pm 0.004 \text{ ppm})$  and lowest in gram (chick pea) bran/hull  $(0.125 \pm 0.002 \text{ ppm})$ . Average mean concentration of Zn in feed ingredients was  $0.507 \pm 0.274$  ppm. Nicholson et al. (1999) found higher concentration of Zn in cattle feeds ingredients than this study, where he reported 91 ppm, 29 ppm, 39 ppm Zn in maize gluten, maize silage and rolled oat and barley, respectively.

**Copper (Cu):** Concentrations of Cu were found within the range from nil to 0.215 ppm (Table 1). Mean concentration of Cu was highest in sesame oil cake  $(0.211 \pm 0.004 \text{ ppm})$  and lowest in gram bran/hull and maize. Average mean concentration of Cu in feed ingredients was  $0.05 \pm 0.058$  ppm. Li et al. (2005) presented the mean Cu concentration in alfalfa hay, haylage, corn silage and corn grain ranges between 3.7 to 6.8 ppm with the standard deviation between 1.2 to 5.3 ppm. Furthermore, the mean for corn grain mix, soybean protein mix ranges between 38.2 to 45.9 ppm with the standard deviation between 46.7 to 72.8 ppm based on Wisconsin dairy rations, which was much higher than our results. In a study, Nicholson et al. (1999) found 10.2, 6.9, 7.6 ppm Cu in maize gluten, cereals and rolled oat and barley, respectively, which was much higher that this results.

Raw materials	Repl.	Zn	Cu	Cr	Cd	Pb
Sesame oil cake	3	$0.683 \pm 0.006$	$0.211 \pm 0.004$	$0.186\pm0.002$	BDL	$0.242\pm0.030$
Wheat bran	3	$0.391 \pm 0.006$	$0.009 \pm 0.002$	$0.097 \pm 0.002$	BDL	$0.188 \pm 0.021$
Gram bran/ hull	3	$0.125\pm0.002$	BDL	$0.134 \pm 0.009$	BDL	$0.216\pm0.447$
Maize	3	$0.194 \pm 0.002$	BDL	$0.173 \pm 0.009$	BDL	$0.210\pm0.014$
Mustard oil cake	3	$0.646\pm0.008$	$0.032 \pm 0.002$	$0.168 \pm 0.004$	BDL	$0.198 \pm 0.021$
Matikalai bran/hull	3	$0.355 \pm 0.003$	$0.022\pm0.005$	$0.180\pm0.006$	BDL	$0.232\pm0.014$
De-oiled rice polish	3	$1.204\pm0.004$	$0.060\pm0.004$	$0.172 \pm 0.003$	BDL	$0.240\pm0.021$
Rapeseed meal	3	$0.606\pm0.006$	$0.048 \pm 0.003$	$0.193 \pm 0.006$	BDL	$0.254 \pm 0.043$
Soybean meal	3	$0.524\pm0.007$	$0.056 \pm 0.006$	$0.190\pm0.004$	BDL	$0.222\pm0.042$
Rice polish	3	$0.569 \pm 0.006$	$0.098 \pm 0.005$	$0.222\pm0.008$	BDL	$0.260 \pm 0.027$
Lentil hull	3	$0.499 \pm 0.006$	$0.060\pm0.002$	$0.226 \pm 0.004$	BDL	0.222 ±0.018
Cotton by products	3	$0.290 \pm 0.003$	$0.001 \pm 0.001$	$0.281 \pm 0.005$	BDL	$0.258 \pm 0.040$
Total		$0.507 \pm 0.274$	$0.050 \pm 0.058$	$0.185\pm0.045$	BDL	$0.228 \pm 0.034$
Minimum		0.124	0.000	0.095	BDL	0.164
Maximum		1.209	0.215	0.286	BDL	0.301

\*BDL= Below Detection Level

**Chromium (Cr):** Concentrations of Cr were found within the range from 0.095 ppm to 0.286 ppm (Table 1). Mean concentration of Cr was highest in cotton by-products (cotton seed hull, cotton) ( $0.281 \pm 0.005$  ppm) and lowest in wheat bran ( $0.097 \pm 0.002$  ppm). Average mean concentration of Cr in feed ingredients was  $0.185 \pm 0.045$  ppm. Concentration of Cr results from the current study is in agreement with the findings of Nicholson et al. (1999), who reported 1.27, 0.26, <0.20 ppm Cr in maize gluten, cereals and rolled oat and barley, respectively. In a study conducted in Bangladesh, mean concentration of Cr were found 10.63 to 218.10 ppm in

protein meal feed (Jothi et al., 2016). Sullivan et al. (1994) have found that, the Cr content of minerals used for feed ingredients may contain Cr ranging from 60 ppm to 500 ppm.

**Cadmium (Cd):** Concentrations of Cr were below detection level (BDL) in all feed ingredients (Table 1). However, Nicholson et al. (1999) found 0.12, <0.10, <0.10 ppm Cd in maize gluten, cereals and rolled oat and barley, respectively. Again, Hossain et al. (2007) found 0.991 to 3.888 ppm cadmium in tannery waste used as feed ingredients.

Lead (Pb): Concentrations of Pb were found within the range from 0.164 ppm to 0.301 ppm (Table 1). Mean concentration of Pb was highest in cotton by-products ( $0.258 \pm 0.040$  ppm) and lowest in wheat bran ( $0.188 \pm 0.021$  ppm). Average mean concentration of Pb in feed ingredients was  $0.228 \pm 0.034$  ppm. Concentration of Pb results from this study is similar with the findings of Nicholson et al. (1999), who reported 2.07, <1, 1.16 ppm Pb in maize gluten, cereals and rolled oat and barley, respectively. Hossain et al. (2007) found higher concentration of lead in tannery waste used as feed ingredients and reported that maximum and minimum concentrations of this element was found 30.114 ppm and 7.577 ppm. In all feed ingredients, mean concentration of Zn was higher than other heavy metals.

#### Concentration of heavy metals in compound commercial beef feeds

The commercial compound beef feeds analyzed were: ACI, gain feed, care feed, Saudia, united feed, provita, index, and IFAEL. The analytical results have been presented as follows (Table 2).

**Zinc** (**Zn**):. Mean concentrations of Zn were found within the range from 0.313 ppm to 1.261 ppm (Table 2). Mean concentration of Zn was highest in Care feed ( $1.255 \pm 0.010$  ppm) and lowest in ACI feed ( $0.314 \pm 0.001$  ppm). Average mean concentration of Zn in compound beef feeds was  $0.787 \pm 0.291$  ppm. The maximum tolerable concentration of zinc for cattle was previously set at 500 ppm (NRC, 2005). However, Nicholson et al. (1999) found 189 ppm Zn in beef cattle nuts/cakes/pellets which were much higher than these findings. Zinc is an essential element needed by your body in small amounts. Without enough zinc in the diet, there could be loss of appetite, decreased immune function, slow wound healing, and skin sores (Gerberding, 2005 & Okoye et al., 2011).

**Copper (Cu):** Concentrations of Cu were found in compound beef feeds within the range from 0.004 ppm to 0.140 ppm (Table 2). Mean concentration of Cu was highest in Teer feed ( $0.138 \pm 0.002$  ppm) and lowest in United feed ( $0.007 \pm 0.004$ ). Average mean concentration of Cu in compound beef feeds was  $0.055 \pm 0.0381$  ppm. Nicholson et al. (1999) found higher concentration of Cu (34.6 ppm) in beef cattle nuts/cakes/pellets. Zhang et al. (2012) found the content of Cu ranged from 2.88–98.08 ppm in cattle feeds.

**Chromium (Cr):** Concentrations of Cr were found within the range from 0.223 ppm to 0.459 ppm (Table 2). Mean concentration of Cr was highest in Saudia feed ( $0.457 \pm 0.003$  ppm) and lowest in Care feed ( $0.226 \pm 0.004$  ppm). Average mean concentration of Cr in compound beef feeds was  $0.304 \pm 0.067$  ppm. Result of Cr is almost similar with the findings of Nicholson et al. (1999), who reported 1.66 ppm Cr in beef nuts/ cakes/ pellets.

Feed	Replication	Zn	Cu	Cr	Cd	Pb
Company	-					
Gain Feed	3	$0.599 \pm 0.002$	$0.060 \pm 0.005$	$0.342 \pm 0.003$	BDL	$0.291\pm0.048$
ACI	3	$0.314 \pm 0.001$	$0.054 \pm 0.001$	$0.341 \pm 0.005$	BDL	$0.264 \pm 0.021$
Care Feed	3	$1.255 \pm 0.010$	$0.014 \pm 0.003$	$0.226 \pm 0.004$	BDL	$0.271\pm0.012$
Teer	3	$0.735 \pm 0.002$	$0.138 \pm 0.002$	$0.251 \pm 0.005$	BDL	$0.256 \pm 0.034$
Saudia	3	$0.568 \pm 0.003$	$0.047 \pm 0.003$	$0.457 \pm 0.003$	BDL	$0.311\pm0.023$
United feed	3	$0.687\pm0.012$	$0.007\pm0.004$	$0.305 \pm 0.007$	BDL	$0.321 \pm 0.024$
Provita	3	$0.853 \pm 0.009$	$0.032\pm0.001$	$0.276\pm0.001$	BDL	$0.299 \pm 0.012$
Index	3	$1.220\pm0.010$	$0.053 \pm 0.003$	$0.288 \pm 0.003$	BDL	$0.309\pm0.009$
IFAEL	3	$0.853 \pm 0.003$	$0.087 \pm 0.004$	$0.256 \pm 0.009$	BDL	$0.343 \pm 0.031$
Total		$0.787 \pm 0.291$	$0.055 \pm 0.0381$	$0.304 \pm 0.067$	BDL	$0.296\pm0.035$
Minimum		0.313	0.004	0.223	BDL	0.218
Maximum		1.261	0.140	0.459	BDL	0.361
Level of sig.		0.000	0.000	0.000	-	0.013
NRC, 2005		500	40	100	10	100
EU, 2003		250	25	-	0.5	5

Table 2. Heavy metals concentrations of commercial compound beef feeds (in ppm ± SD) in DM basis

\* BDL = Below Detection Limit

**Cadmium (Cd):** Concentrations of Cr were below detection level (BDL) in all compound beef feeds (Table 2). A survey of Cd in cattle feed and cattle manure reported the presence of Cd in cattle feed in farms of different herd sizes, with the mean value between 0.38 to 2.31 ppm in Northeast China (Zhang et al., 2012). However, other reports suggested that mean value of Cd concentration in cattle feed at 2.79 ppm in Beijing and Fuxin, China (Li et al., 2010). Elliott et al., (2017) reported that 21% ruminant feed contain Cd content over the limit of EU. He found maximum 68 ppm Cd in ruminant feed which was higher than this result.

Lead (Pb): Concentrations of Pb were found in compound beef feeds within the range from 0.218 ppm to 0.361 ppm (Table 2). Mean concentration of Pb was highest in IFAEL feed ( $0.343 \pm 0.031$  ppm) and lowest in Teer feed ( $0.256 \pm 0.034$  ppm). Average mean concentration of Pb in compound beef feeds was  $0.296 \pm 0.035$  ppm. Results of Pb from the current study is in agreement with the findings of Nicholson et al. (1999), who reported <1.00 ppm Pb beef nuts/ cakes/ pellets. Elliott et al., (2017) reported that 16% ruminant feed contain Pb content over the limit of EU. He found maximum 645 ppm Pb in ruminant feed which was higher than this

result. Li et al. (2005) reported that the mean concentration of Pb in the feed samples were all below 1 ppm with the exception of mineral premixes, whose mean value was 2.857 ppm with the standard deviation being 2.483 ppm.

### Concentration of heavy metals in organs and beef

The organs and beef analyzed were: heart, kidney, liver, lung and muscle. The analytical results have been presented as follows (Table 3):

**Zinc (Zn):** Concentrations of Zn were found within the range from 3.944 ppm to 16.218 ppm (Table 3). Mean concentration of Zn was highest in heart (6.059  $\pm$  3.362 ppm) and lowest in beef muscle (15.883  $\pm$  0.438 ppm). Makridis et al., (2012) reported Zn content in muscle, liver, kidney at the level of 38.3 ( $\pm$  2,6), 23.5 ( $\pm$  2.5), 13 ( $\pm$  2.5) ppm respectively in cow which was higher than this result.

**Copper (Cu):** Concentrations of Cu were found in organs and beef within the range from 2.228 ppm to 218.801 ppm (Table 3). Mean concentration of Cu was highest in liver (172.665  $\pm$  58.342 ppm) and lowest in muscle (2.510  $\pm$  0.267). Copper concentrations are highest in the liver, and copper in liver increases in animals fed high dietary copper (NRC, 2005). Increased incidence of copper toxicosis in cattle has been reported recently by the Veterinary Laboratories Agency (VLA) in England (Bidewell et al., 2000; VLA, 2001) Makridis et al., (2012) found Cu content in muscle, liver, kidney at the level of < 0.02 ppm, 51 ( $\pm$  7.4) ppm, 2.3( $\pm$  0.3) ppm in cow which was much lower than our study. Copper is an essential element for all known living organisms including humans and other animals at low levels of intake. However, exposure to higher doses can be harmful. Long-term exposure to copper dust can irritate your nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea (Gerberding, 2004; Okoye et al., 2011).

**Chromium (Cr):** Concentrations of Cr were found in organs and beef within the range from 0.086 ppm to 0.986 ppm (Table 3). Mean concentration of Cr was highest in heart  $(0.555 \pm 0.451 \text{ ppm})$  and lowest in kidney  $(0.164 \pm 0.039 \text{ ppm})$ . Makridis et al., (2012) found Cr content less than 0.02 ppm in muscle, liver, kidney which was lower than our study. Furthermore, studies done by Miranda et al. (2009) have shown that accumulation of Cr in animal tissues was generally low and within the normal range.

**Cadmium (Cd):** Concentrations of Cd were found in organs and beef within the range from 0.002 ppm to 3.145 ppm (Table 3). Mean concentration of Cd was highest in kidney (2.339  $\pm$  0.712 ppm) and lowest in heart (0.003  $\pm$  0.001 ppm). Cadmium accumulation is greatest in the kidney, followed by liver, testes, pancreas, and spleen. Muscle and bone do not accumulate cadmium at high levels (Table 3). Makridis et al., (2012) reported Cd content in muscle, liver, kidney at the level of 1.3 ( $\pm$  0.3), 1.4 ( $\pm$  0.3), 1.8 ( $\pm$  0.4) ppm respectively in cow which were higher than our findings. Feeding heifers a diet containing 5 ppm cadmium for more than a year before parturition and throughout lactation did not result in significantly elevated milk cadmium levels during any part of lactation (Van Bruwaene et al., 1982). Regardless of the level of cadmium fed, the concentration of cadmium in meat, milk, and eggs is always lower than the level in the diet that the animal consumed (on a dry matter versus dry matter basis). However, levels in kidney and liver are always considerably higher than the levels in the diet and these tissues magnify environmental cadmium (Morcombe et al., 1994). Cadmium toxicity has been linked to prostate cancer and cancer in liver, kidney and stomach (Waalkes, 2000).

Meat (As fresh)	No. of sample	Zn	Cu	Cr	Cd	Pb
Heart	3	$6.059 \pm 3.362$	$19.385 \pm 2.381$	$0.555 \pm 0.451$	$0.003 \pm 0.001$	$0.084\pm0.046$
Kidney	3	$9.984 \pm 1.358$	$12.556 \pm 1.634$	$0.164 \pm 0.039$	$2.339 \pm 0.712$	$0.364 \pm 0.202$
Liver	3	$14.776 \pm 0.816$	$172.665 \pm 58.342$	$0.199 \pm 0.091$	$0.299 \pm 0.221$	$0.263\pm0.056$
Lung	3	$11.567 \pm 2.625$	$7.777 \pm 1.164$	$0.512 \pm 0.018$	$0.024 \pm 0.005$	$0.278\pm0.089$
Muscle	3	$15.883 \pm 0.438$	$2.510\pm0.267$	$0.232\pm0.023$	$0.014\pm0.009$	$0.094\pm0.046$
Min.		3.944	2.228	0.086	0.002	0.032
Max.		16.218	218.801	0.986	3.145	0.583

 Table 3. Heavy Metal Concentrations in Organs and Beef

Lead (Pb): Concentrations of Pb were found in organs and beef within the range from 0.032 ppm to 0.583 ppm (Table 3). Mean concentration of Pb was highest in kidney ( $0.364 \pm 0.202$  ppm) and lowest in heart ( $0.084 \pm 0.046$  ppm). Accumulation of lead in tissues is dependent upon the dose and the length of exposure. These relationships have been extensively detailed in rat models (IPCS, 1995; ATSDR, 1999). In acutely sick cattle poisoned by licking the remains of storage batteries burned and left in a pasture, levels of lead in milk averaged 0.08 mg/L and in the muscle ranged from 0.23 to 0.5 ppm (wet weight basis) compared to normal levels of below 0.005 and 0.01 ppm, respectively. Very high concentrations were found in the kidneys, with a range of 70–330 ppm (Oskarsson et al., 1992). In cattle grazing near a lead smelter, the tissue lead levels were highest in cattle grazing near the smelter and decreased with distance (Neuman and Dollhopf, 1992). Makridis et al. (2012) reported Pb content in muscle, liver, kidney at the level of < 0.02, < 0.02, < 0.02, < 0.02,  $(\pm 3)$ , ppm respectively in cow which was slightly lower than this study. Lead exposure has been associated with elevated blood pressure and hypertension (Martin et al., 2007).

# Conclusions

In this study we evaluated the heavy metals (copper, zinc, chromium, lead, cadmium) in the twelve samples of feed ingredients used in cattle feed formulation and nine commercial beef feed in Bangladesh. We also evaluated different organs and muscle of beef cattle to find the residue of heavy metals in meat. The content of five elements (Cu, Zn, Cr, Pb, Cd) of each heavy metal were found in feed and meat and evaluated according to EU & NRC standards which were below the maximum residue limit (MRL) level. Only cadmium was not found in commercial beef feed and feed ingredients.

#### **Conflicts of Interest**

The authors declare that there are no potential conflicts of interests.

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