Animal Review
2016 Vol. 3, No. 2, pp. 52-58
ISSN(e): 2409-6490
ISSN(p): 2412-3382
DOI: 10.18488/journal.ar/2016.3.2/101.2.52.58

EFFECT OF ENVIRONMENTAL POLLUTION ON PRODUCTION AND METABOLIC PROFILE IN BUFFALO COWS

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ABSTRACT

The aim of this study was to verify the effects of environmental impact caused by dioxin on milk production and metabolic profile in the buffalo cows. The authors analyzed some representative blood parameters related to their different metabolisms, the amount of zinc in blood samples, and milk production parameters, on 160 buffalo cows raised in four farms located in areas with a different environmental impact. Urea, glucose, creatinine, AST (aspartate aminotransferase), ALT (alanine aminotransferase) and zinc contents were determined on serum samples. Milk samples from each buffalo were collected. Daily milk yield and milk composition were significantly different among farms. No difference in milk protein content was found. Urea content, was higher. Glucose concentrations were significantly lower than in farms located in an area contaminated by dioxin. Creatinine values were normal. AST and ALT values were slightly higher than normal. So far, there are not studies on the effect of dioxin on production and metabolic profile in buffalo cows. This study indicates that buffalo breeding herds exposed to dioxins would induce damage in the hepatic parenchyma cells as a result of animal welfare. Accordingly, the effects of environmental pollutants may expose the animals to several infections and diseases, which could affect not only the production traits, but also the qualitative features of their products devoted to human consumption.

Keywords: Dioxin, Metabolic profiles, Milk quality, Buffalo cows, Polychlorinated dibenzo-para-dioxins/ furans, Polychlorinated biphenyls.

Received: 13 October 2016/ **Revised:** 4 November 2016/ **Accepted:** 9 November 2016/ **Published:** 14 November 2016

Contribution/ Originality

Considering the increasing economic relevance of the Buffalo and considering the insufficiency information in the literature on the influence of micro pollutants on the buffalo, this work has focused the attention on the influence the environmental impact on buffalo health and on their production. It is important to remember that the animal is closely linked to the area, monitoring animal's means monitoring the breeding environment.

1. INTRODUCTION

The increasing economic relevance of the Italian Mediterranean Buffalo is due to the lack of milk production quotas in the European Union and especially to the high market demand of buffalo "mozzarella" cheese, whose price is more than double than cow "mozzarella".

Livestock animals represent a crucial point in the food chain, as they transform vegetable matter in animal protein. Since the animal populations are closely linked to the area, monitoring animals means monitoring the breeding environment, especially in case of natural pasture or vegetal products growing in the same territories.

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Usually animals are kept on paddocks and are mainly fed with *unifeed* during lactation and with pasture in case of dry cows and heifers. The Italian buffalo cow produces on average 2150 Kg of milk per lactation, containing 8.28 and 4.74% of fat and protein respectively. According to Food and Agricultural Organization (FAO) [1] in Italy the buffalo cow population increased from 182,000 heads in 2000 (68% female) to 307,100 heads in 2009 (63% female) and the buffalo milk production increased from 135,100 tonnes in 2000 to 210,000 tonnes in 2009. However, the knowledge of the effects of environmental pollution on milk production for this species is limited.

Recently, environmental pollution received increasing attention from politics, both at national and international level, as a result of the increasing awareness of social and health aspects involved in this issue. In particular, during the last two decades, a large environmental devastation occurred on several Italian regions, because of the burning of municipal waste releasing significant quantities of high pollutant contaminants.

Iron and steel manufacturing factories are responsible for the emission of harmful pollutants, such as dioxin, sulphur oxides, nitrogen oxides, hydrocarbons etc. that can cause genotoxic and carcinogenic effects [2-5].

Dioxin are polyhalogenated aromatic hydrocarbons, highly toxic and persistent, present at low level in the air, soil, water, feed as well as in foods such as dairy products [6].

A dioxin and furan concentration analysis were published in 2008 by ARPAC (Regional Agency for Environmental Protection of Campania) [7] allowing the identification of three contaminated areas, scored as high (>1.4 ng/Kg of dioxin and furan in the soil), medium (<1.4 and >1.0 ng/Kg) and low (<1.0 ng/Kg) impact.

Therefore, fodder is contaminated by toxic chemicals and the animal eats large quantities of these contaminants in feed, possibly damaging their own health and that of the human consumer of their products.

Some metals act as catalysts for dioxins and furans synthesis [8] actually providing a surface for a rapid formation of these compounds. According to the WHO (World Health Organization), metals of most immediate concern are chromium, zinc, iron, mercury and lead; zinc is a trace element essential in quantities exceeding any other trace element, besides iron, it is known for its protective properties against diseases and is also very important for immune system. considering the dual role (positive and negative) and toxicity of metals we made a preliminary investigation quantifying the blood parameters representative of the energetic and protein metabolisms, the zinc amount in blood samples, and the milk parameters of buffaloes reared in farms located in areas having different environmental impact.

2. MATERIAL AND METHODS

2.1 Farm Selection and Animals

The present research involved 160 lactating buffaloes in four farms (40 per farm), during 90 days of lactation. Two of them (A and B) were located in an area of low environmental impact (near Salerno – Italy) and the other two (C and D) were in an area of high environment impact (near Caserta – Italy) (Table 1).

Table-1. Dioxin levels in the milk mass obtained from buffalo cows of groups A, B, C and D

	Buffalo Farms			
	A	**B**	**C**	**D**
WHO-PCDD/F-TEQ (pg./g of fat)	N.D.	N.D.	17.00 (3.0)	15.90(3.0)
WHO-PCB-TEQ (pg./g of fat)	N.D	N.D.	4.79	4.50
WHO-PCDD/F-PCB-TEQ (pg./g of fat)	1.6 (6.0)	1.3 (6.0)	21.79 (6.0)	20.57 (6.0)

WHO: World Health Organization; TEQ: Toxic Equivalents; PCDD/F: polychlorinated dibenzo-*para*-dioxins and furans; PCB: polychlorinated biphenyls; N.D.: not detected Maximum levels legally admitted are reported in parenthesis. Data are expressed as pg. per g of milk fat

Farms were chosen on the basis of the Agenzia Regionale per la Protezione Ambientale della Campania [7]. A and B farms showed dioxin, PCDD/F (polychlorinated dibenzo-para-dioxins/ furans) and PCB (polychlorinated biphenyls) levels lower than 2 pg./g of milk fat, while in C and D farms these values were higher than 3 pg./g of milk fat (maximum permitted level reported by the Commission Directive *2002/69/*EC of 26 July 2002).

Lactating buffalo cows were randomly sampled in farms. In each farms groups were homogeneous in parity, Body Condition Score (BCS) and health condition.

The analysis of dioxin levels in milk samples was carried out by accredited Italian laboratories in according to the standards of national health agency.

In each of the farms, animals had *ad libitum* access to *unifeed* containing vitamins and minerals**.** Farms were also chosen according to their management: as shown in Table 2, farms A and C, and farms B and D were also similar in ration characteristics, both in terms of forage/concentrate ratio (F:C), and chemical-nutrition characteristics. Animals were raised in open paddocks in all farms.

Table-2. Feed and chemical composition of the diets (as percent of dry matter)

		Buffalo cows farm			
		A	**B**	**C**	**D**
Mais silage	%	62.90	37.50	46.20	36.00
Wheat straw	%	14.30	--	--	--
Concentrate	%	8.00	43.90	20.5	44.00
Barley straws	%	--	18.60	12.80	--
Ryegrass hay	%	--	--	20.50	20.00
F:C ratio		**68.5 : 31.5**	**44.8 : 55.1**	**65.6 : 34.4**	**48.1 : 51.9**
Chemical composition					
Crude protein	%DM	13.70	16.82	13.38	18.65
Ether extract	%DM	3.51	3.89	3.15	3.92
Crude fiber	%DM	20.73	18.37	23.86	16.11
Ash	%DM	7.68	8.36	7.99	8.48
NDF	%DM	37.52	28.79	44.21	24.49
ADF	%DM	22.93	18.73	27.80	14.53
NSC	%DM	37.58	42.15	31.72	44.47
Milk Forage Unit	Kg^{-1DM}	0.79	0.79	0.74	0.83

NDF, neutral detergent fiber; ADF, acid detergent fiber; NSC, nonstructural carbohydrates

In each farm, the following mineral integrations, gr per Kg of concentrate, were used (table 3).

Table-3. Mineral integration in each farm (gr per Kg of concentrate)

	MnO	**$FeCO_3$**	**CuO**	**$CoSO_4$•$7H_20$**	**KI**	**ZnO**	**Na_2SeO_3**
A	100.0	80.0	20.0	1.3	1.0	160.0	0.4
B	190.0	120.0	30.0	2.0	2.0	250.0	0.1
C	160.0	140.0	45.0	3.0	5.0	330.0	0.5
D	150.0	80.0	20.0	1.3	1.0	160.0	0.4

MnO, manganous oxide; ADF, $FeCO_3$Iron triossocarbonate; CuO, cupric oxide; $CoSO_4$•$7H_20$, Cobalt Sulfate Heptahydrate; KI, potassium iodide; ZnO, zinc oxide; Na_2SeO_3, Sodium selenite.

2.2 Determination of Metabolic Profiles and Zinc

Urea, glucose, creatinine, AST and ALT contents were determined on serum samples, collected once a week from each buffalo from the jugular vein by the Vacutainer method (12 samples per animal per farm).

Blood parameters were determined on serum by using Reflotron (Boehringer, Ingelheim, Germany), measuring urea, creatinine, ALT, AST and glucose (Table 4). The reference metabolic values are those reported by Commission Scientific Association of Animal Production (ASPA) [9] and shown in the legend.

Zinc content was determined on serum samples by atomic absorption spectrometry. Milk samples from each buffalo were collected fortnightly for 90 days (6 samples per animal per farm), during the morning and afternoon milking, in order to evaluate milk composition (fat and protein) by infrared method (Milko Scan 133B Foss Electric, Hillerød, DK, calibrated with the appropriate buffalo standard). The numbers of somatic cells on each milk samples were determined by a fluoro-optometric method (Fossomatic 90). Somatic cell count (SCC) was log10-transformed before analysis because it was not normally distributed.
The samples for determining all parameters were processed in triplicate.

Table-4. Metabolic profile

		Buffalo cows, farms			
		A	**B**	**C**	**D**
		means ± SD	means ± SD	means ± SD	means ± SD
Urea	mg/dl	54.62 ± 8.12	56.40 ±9.41	50.90 ± 7.67	50.62 ± 8.25
Glucose	mM/l	3.34 ± 0.26 **b**	3.36 ± 0.95 **b**	2.41 ± 0.64 **a**	2.34 ± 0.71 **a**
Creatinine	µM/l	137.4 ±16.79	136.27 ± 18.36	127.55 ± 16.57	129.78 ± 15.32
AST	UI/l	157.0 ± 25.87 **b**	158.08 ± 26.13 **b**	165.77a ± 27.67 **a**	161.87 ± 29.92 **a**
ALT	UI/l	63.49 ± 8.58 **b**	63.85 ± 9.24 **b**	66.15 ± 10.73 **a**	67.53 ± 9.15 **a**
Zinc	µM/l	8.59 ± 0.99 **a**	8.65 ± 0.93 **a**	7.62 ± 0.94 **b**	7.55 ± 0.91 **b**

a, b is significant at the 0.01 level

Reference values ASPA Commission: urea: 30 – 45 (mg/dl); Glucose: 4.1 – 4.5 (mM/l); Creatinine: 120 – 155 (µM/l); AST: 120-145 (UI/l); ALT: 30-37(UI/l); Zinc: 9-12 (µM/l).

2.3. Statistical Analysis

ANOVA was carried out to evaluate significant differences on milk production and milk quality among farms [10].

3. RESULTS AND DISCUSSION

3.1. Analysis of Metabolic Profile and Zinc Content

The ARH cell receptor (Aryl Receptor Hydrocarbon) is located in both the cytoplasm and nucleus of cells. Dioxins bind to this receptor diffusing into the cells, which are equipped with ARH receptors. The dioxin-receptor complex binds to a nuclear protein called ARNT (AR Nuclear Translocator). Thus forming a complex similar to some sequences of DNA results in:

- interactions with growth factors;
- activity on the metabolism of some hormones;
- activity on the mechanisms of differentiation and cell division;
- hormone disturbances.

According to these activities the study of the influence of different levels of dioxin on some proteins and energetic metabolisms has been widened.

Table 4 shows the average of metabolic profile on blood samples during the trial.

Urea content appears to be slightly higher than normal values in all tested farms. Similar values were found by Campanile, et al. [11]. The values we observed appear to be related to those that we observe when the buffalo recycles endogenous urea to maintain protein balance, which during chronic stress can induce inflammatory mechanisms in organs and tissues.

In all tested buffalos, glucose values were significantly lower than the physiological values. This attitude may be due to both an energy deficit, to which animals can be subjected, and a prolonged stress. In ruminants the blood glucose derives mainly from propionic acid produced in the rumen, and in a smaller extent from amino acids or from direct ingestion of starch.

The latter is the most important action, which explains the huge variety of effects induced by dioxins. Actually, two glands, adrenal medulla and pancreas, act keeping the blood glucose at a constant, balanced level, hence optimizing its use by many kinds of cells. The former produces adrenaline, a substance causing the raising of the blood glucose level, while the latter produces insulin, an hormone that helps "clearing" the blood glucose so that it enters in cells of several organs. Strongly stressful situations stimulate the adrenal medulla to produce excess adrenaline, which in turn causes a rise in blood glucose. Consequently, blood glucose level will rise, resulting in a continuous insulin requirement by the pancreas. Thus one can achieve pancreatic stimulus such that even after small increases in blood glucose, a large insulin response will result, causing an excessive lowering of the glucose level according to Sood, et al. [12]. These authors reported that chronic stress is associated with hormonal changes that are known to affect multiple systems, including the immune and endocrine systems.

In farms located in high environmental impact areas, glucose concentration was significatively lower than that achieved in those localized in high impact areas.

Consequently, in C and D farms the environmental stress likely influenced the glucose levels, reducing them ($P<0.01$).

The creatinine values were normal.

The high levels of aspartate aminotransferase and alanine aminotransferase are pathognomonic of a liver disturbance. Since the aspartate transferase enzyme is localized in the cytoplasm of biliary canals, it can be inferred that the damage is present in the parenchymatous cells. This cytolysis may not be directly linked to the environmental impact, but rather to a lack in glucose and a higher level of ammonia, or to an inflammatory reaction, that causes a reduction of circulating zinc.

Actually the highest values of ALT and AST were observed in farms located in areas of higher environmental impact, these differences being highly significant ($P<0.01$).

Terzano, et al. [13] reported that different management and different environment influence glucose, NEFA (Non Esterified Fatty Acids), urea, AST and ALT concentrations.

Zinc values recorded in A and B farms were very close to their physiological levels, while in C and D farms the lacking of zinc is even more evident. These values can likely be related to the different pollution level to which different farms are exposed, and are consistent to the other measured blood parameters. This confirms the suspected effect of the environmental impact on different metabolisms, especially considering that mineral integration in high-impact farms is greater than in A and B farms.

Xu, et al. [14] showed that integration of vitamin E can reduce the effects of dioxin on the reproductive system in experimental animals.

Spagnuolo, et al. [15] reported that the higher levels of products of oxidative damage (PC - protein-bound carbonyls, N-tyr - nitro-tyrosine and LPO - hydroperoxides), and decreased levels of antioxidants were detected in buffalo cows exposed to dioxins. This clearly indicates that breeding in dairy herds exposed to dioxins promotes plasma protein and lipid oxidation. The above-reported differences in the blood redox condition between the analyzed groups would indicate that exposure to dioxins impairs the non-enzymatic component of the antioxidant defense system, and that metabolic processes associated to dioxin detoxification might induce and/or enhance oxidative protein and lipid damage.

3.2. Analysis of Milk Quality

Table 5 shows the average of milk yield, percentage of fat and protein, and SCCt (somatic cell count transformed $\log_{10}$) values of the milk collections during the trial.

Table-5. Daily milk yield and composition; number of transformed somatic cells

	Buffalo cows farm			
	A	**B**	**C**	**D**
	means ± SD	means ± SD	means ± SD	means ± SD
M.Y. (Kg)	16.4 B ± 1.33	13.7 D ± 1.17	17.6 A ± 1.38	15.5 C ± 1.25
Fat (%)	9.06 A ± 2.01	8.71 B ± 1.98	8.18 D ± 2.10	8.43 C ± 1.80
Protein (%)	4.65 ± 0.94	4.71 ± 0.93	4.69 ± 0.87	4.67 ± 0.91
SCC transformed	2.07 C ± 0.42	2.00 D ± 0.50	2.15 B ± 0.47	2.25 A ± 0.45

A, B, C and D is significant at the 0.05 level

Daily milk yield, fat content and SCCt resulted significantly different among farms ($P<0.05$), while no difference was found in milk protein content.

4. CONCLUSIONS

The buffalo, for its characteristics of rusticity, is probably able to better tolerate the stress factor represented by micro pollutants. This result indicates that buffalo breeding herds exposed to dioxins would induce damage in the hepatic parenchyma cells by consequences on animal welfare.

Accordingly, the effects of environmental pollutants may render animals susceptible to several infections and diseases, which could affect not only the production traits, but also the qualitative features of their products devoted to human consumption.

However further investigation is necessary to assess the long-term effects on the productions.

Funding: The study was supported by the Italian National Research Council under DG.RSTL.083.001 We are grateful to Giuseppe Grazioli for his technical assistance.

Competing Interests: The authors declare that they have no competing interests.

Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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