

Clenbuterol Residues in Bovine Feed and Meat

¹Silvia Denisse Peña Betancurt, ²Alejandro Córdova-Izquierdo

³Antonio Uribe and ¹Aldo M. Michel

¹Toxicology Laboratory, Department of Agricultural and Animal Production,
University Autonomous Metropolitan-Xochimilco, Uam-Xochimilco, Mexico

²Department of Agricultural and Animal Production, Health and Animal Welfare, Autonomous Metropolitan
University-Xochimilco Calz, del Hueso 1100 Col, Villa Quietud ZP. 04960, Mexico, DF

³Autonomous University Tamaulipas, Mexico

Abstract: The aim of this study was to measure the β -agonist, clenbuterol in bovine feeds and meat in North and South of Mexico. Clenbuterol is used illegally as growth promoter in animal feeding in farms animals due to muscle hypertrophy and lipolysis effect. In Mexico, there is a suspected in their unlicensed use in certain commercial feedlots. Total 16 food samples and 90 samples of meat were analyzed by 2 commercially clenbuterol enzyme immunoassays kits. The samples were collected during 2006. Approximately, 75% of the animal feed samples and 16.6 % of meat analyzed were found positives in levels ranged 2525-53787 ppt and 0.1-2.3 $\mu\text{g kg}^{-1}$. These information indicate that the abuse of clenbuterol during the last 5 years is not declining in some farms of Mexico with a health human risk.

Key words: Clenbuterol, residues, feed, meat, bovine

INTRODUCTION

Clenbuterol is used as repartitioning agent in meat production in doses approximately 10 times higher than the therapeutic dose ($1.5 \mu\text{g kg}^{-1}$ during 10 days in cows no lacting or $0.5 \mu\text{g kg}^{-1}$ in a single dose (Millar *et al.*, 1988; Kuiper and Noordam, 1998). The limit maximum residue (RML) in meat ranged from were $0.5\text{-}0.2 \mu\text{g kg}^{-1}$ and 10,000 ppt for animal feed. Numerous reports on food poisoning throughout Europe prompted the regulatory as growth promoter (Prezelj *et al.*, 2003). The occurrence of 4 cases of acute food poisoning involving a total of 50 people, due to the ingestion of liver of lamb and bovine meat containing residues of clenbuterol were notified in Portugal by Barbosa *et al.* (2005), sporadic cases of human intoxications in Mexico, since 2002 in Aguascalientes were 20 people dawn intoxicates until February 2007 with 170 cases and state of Jalisco that actually detected 31 human intoxications by ingestion of contaminated meat, however, exist a prohibition of clenbuterol since 2000 (NOM, 2002).

In feeding of bovine is common the use of supplementation with β -agonists by to increase protein deposition and decrease fat in domestic animals. The β -agonist clenbuterol is used by some farmers of illegal way (Shiavone *et al.*, 2004).

Several commercially available immunoassay kits have been developed to detect the beta adrenergic agonist clenbuterol HCl, based mainly on chromatography and immunoassay screening. The positive analysis for clenbuterol in meat is 0.2 ppb and 10, 000 pg g^{-1} for feed. It has been showed that some kits for clenbuterol may detect other cross-reacting beta agonists (cimaterol, salbutamol or terbutaline).

The purpose of this research was to confirm the use of clenbuterol in some farms on North and South bovines in Mexico.

MATERIALS AND METHODS

A sampling of foods was made totally at random in farms with intensive production located in South of Mexico during January to October 2006. The meat was collected in 9 cities of North of Mexico in a meat shop. The samples were ground and weight before the clenbuterol determination. Two commercial immunoassay purchased were used by clenbuterol detection in feed and meat, 1 of Neogen and other by R-Biopharm, this last by direct analysis. The sensitivity and linearity of response of the assays proved appropriate to the determination of clenbuterol. The both test operates on the basis of competition between the enzymes conjugate. Is read a

Corresponding Author: Alejandro Córdova-Izquierdo, Department of Agricultural and Animal Production,
Eco-development of Animal Production, Health and Animal Welfare, Autonomous Metropolitan
University-Xochimilco Calz, Del Hueso 1100 Col, Villa Quietud Zp. 04960, Mexico, DF

microwell reader to yield optical densities. The standard curve is formed for calculate the concentration of clenbuterol. The sensitive is $0.1 \mu\text{g kg}^{-1}$ and they have a low cross-reactivity.

RESULTS AND DISCUSSION

The results of feed to come south bovine farms were ranged from 2525-53787 ppt with a mean to 30445.5 ppt. In meat tissue the maximum level was 2.30 and the minimum 0.1 and a ranged from 0.13 ppb. These results are showing in Table 1 and 2.

The values obtained of clenbuterol in feed showed in this research over many times higher than the current therapeutic dose recommended so the illegal used like anabolic effect is suggested. The 16.6% positives samples from meat analysis were low levels, if it compares the 37.5% positives of liver samples obtained in 2002. However, it is important to make a confirmatory procedure due to potential source of error like a sample clean up of feed samples, as mentioned by Wang and Shen (2007). The both analytical procedure used to screening yielded positives results. The R-Biopharm kit was simple in feed samples and the Neogen kit for meat samples is rapid and economical, too; however, the use of commercial kits to quantitative clenbuterol might lead to an overestimation is for that the screening analysis should be confirmed by gas chromatography-mass spectrometric analysis (GC-MS). The maximum residue limit (LMR) approved for clenbuterol in tissues is 0.2 ppb and 10,000 ppt for feeds, the concentration range as measured in this research would exceed these limit its important to note that only 4 of 9 cities survey were free of clenbuterol residues.

Table 1: The results of clenbuterol found in bovine feeds in south of Mexico

Feed samples	Calculated ppt	Dilution factor	Samples P/N
M1	3080	38500	P*
M2	4282	53525	P*
M3	3651	45637	P*
M4	4303	53787	P*
M5	3646	45575	P*
M6	-	-	-
M7	-	-	-
M8	1054	13675	P*
M9	309	3862	N**
M10	974	12175	P*
M11	202	2525	N**
M12	2365.44	29565	P*
M13	1908.00	23850	P*
M14	2052.72	25662	P*
M15	3737.70	46712	P*
M16	2495.10	31187	P*
Mean	2471.92	30445.5	
Positive % samples			75

*Limit 10,000 ppt = positive

Table 2: Clenbuterol detected in muscle tissue between january-october, 2006 in north of Mexico

Cities	Tampico	Mante	Aldama	Victoria
No. samples	10.0	10.0	10.0	10
No. positives	4.0	3.0	5.0	0
Positives (%)	40.0	30.0	30.0	0
Mean*	0.31	0.05	0.39	0

Cities	S. Fernando	Jiménez	Matamoros	Rey	N.L.
No. samples	10.0	10.0	10	10	10
No. positives	2.0	2.0	1	0	0
Positives (%)	20.0	20.0	10	0	0
Mean*	0.23	0.17	0	0	0

*Values from $\mu\text{g kg}^{-1}$

CONCLUSION

In conclusion, 75% of feed samples tested were positive to β -agonist clenbuterol in a levels allowed for the Mexican regulation is suggest a control of their abuse for farmers in South of Mexico. Total 16.6% of the meat tissue from nine cities from North of Mexico, were clenbuterol positives in a low levels. However, any residue is unacceptable. The both enzyme-linked immunosorbent assay used for detection of clenbuterol probed were easy but a confirmatory procedure is always suggested.

REFERENCES

- Barbosa, J., C. Cruz and J. Martins, 2005. Food poisoning by clenbuterol in Portugal. *Food Addit. Contam.*, 22 (6): 563-566.
- Kuiper, H.A. and M.Y. Noordam, 1998. Illegal use of b-adrenergic agonists: European community. *J. Anim. Sci.*, 76:195-207.
- Millar, M.F., M.E. Garcia and P.A. Coleman, 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol fed heifers. *J. Anim. Sci.*, 66 (12): 123-127.
- NOM-EM-015-ZOO, 2002. Norma Oficial Mexicana de emergencia. Technical specification to control use of β -agonist in animal feeds.
- Prezelj, A., A. Obreza and S. Pecar, 2003. Abuse of clenbuterol and its detection. *Curr. Med. Chem.*, 10 (4): 281-290.
- Shiavone, A., M. Tarantola and G. Perona, 2004. Effect of dietary clenbuterol and cimaterol on muscle composition, β -adrenergic and androgen receptor concentrations in roiler chickens. *J. Anim. Phys. Anim. Nutr.*, 88 (3-4): 94-100.
- Wang, J.P. and J.Z. Shen, 2007. Immunoaffinity chromatography for purification of salbutamol and clenbuterol followed screening and confirmation by ELISA and GC-MS *Food Agric. Immun.*, 18: 107-115.