KRMIVA

DETERMINATION OF ACTIVE SUBSTANCES IN MEDICATED FEEDSTUFFS

ODREĐIVANJE AKTIVNIH TVARI U LJEKOVITIM KRMIVIMA

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Original scientific paper - Izvorni znanstveni članak
Received - Primljeno: 8. april - travanj 2010.

SUMMARY

From January 1, 2006 antibiotics, other than coccidiostats and histomonostats, may be used only in medicated feedstuffs. Medicated feedstuffs are feedstuffs containing an authorized medicated premix or an authorized intermediate product and they are intended to be fed to animals without any further processing. As a result of this, specific tests are required to study the quality of the medicated premixes and feedstuffs. The conditions for the production, marketing and use of medicated feedstuffs are clearly specified. The active substance content in medicated feedstuffs is determined in a description. The official control includes the quantitative determination of the active substance content in 1 g medicated feedstuff and homogeneity of this kind of feed. The criterion for the assessment of homogeneity is the coefficient of variation (CV) ≤ 15%. The official control of medicated feedstuffs includes the microbiological analysis of the following substances; amoxicillin, chlortetracycline, doxycycline, lincomycin, tylosin and tiamulin. Medicated feeds, intermediate products, medicated premixes and cleaning mixtures were analysed. In four years (2006 – 2009) 1454 samples were examined. Among the samples of medicated feedstuffs 116 (9.75%) were non-compliant with the declaration or the homogeneity was incorrect – value of the CV was ≥ 15%. Of the 134 cleaning mixtures 34 (25.37%) ware positive, it means that they contained residues of an active substance used in medicated feedstuff.

Key words: active substances, medicated feedstuffs

INTRODUCTION

Antibiotics are widely used in veterinary medicine for therapeutic, metaphylactic or prophylactic treatment of bacterial infections in farm animals. The rationale veterinary use of antibiotics is to protect animal welfare, to prevent epidemic spread of infectious animal diseases, to provide high efficiency of animal production and to prevent the transfer of zoonoses from animals to the human population (Ungemach et al. 2006). Medicated premixes are intended for oral administration following incorporation in animal feedstuffs. Medicated feedstuff is defined as “any mixture of a veterinary medicinal

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product or products and feed or feeds which is ready prepared for marketing and intended to be fed to animals without any further processing, because of its curative or preventive properties or other properties as a medicinal product" (Council Directive 90/167/EEC). Medicated feedstuffs are those that contain veterinary medicinal products on the advice of a veterinary surgeon. The conditions for the production, marketing and use of medicated feedstuffs are clearly specified (Council Directive 90/167/EEC; Law of 22 July 2006). Medicated feedstuffs may be prepared only from premixes which have been authorized under the suitable acts. Currently in Poland there are a few dozen medicated premixes authorized. The type, nature and quality of the feedstuff into which the medicated premix will be incorporated should be described. The categories of animals to which it may be fed should be indicated. The concentration of the active ingredient in the feedstuff must be stated in terms of mg/kg feed and related to the dose in mg/kg bodyweight. The reduction of an active substance content can cause low effectiveness of drugs and increasing occurrence of antibiotic resistance in organisms. However, the overdose can cause chronic or acute poisoning (intoxication). The residues of antibiotics and other antibacterial substances can also occur in food and the amount of drugs larger than is expected get to the environment (Rybarczyk and Gajęcki, 1996).

The evidence of homogeneity should be presented to demonstrate that adequate mixing of the active ingredient in the final medicated feed is likely to be achieved. Medicated feeds are frequently transported over long distances by different means of transport and unless otherwise justified a study should be carried out to demonstrate that there is no physical separation of the medicated premix from the feedstuff during transport which could result in loss of homogeneity. The criterion for the assessment of homogeneity is the coefficient of variation (CV) ≤ 15%. The value of CV was determined based on results from the validation of the methods.

Taking these facts into account there is a necessity to control the active substances content in medicated premixes and feedstuffs. The study was undertaken in accordance with the national monitoring programme for animal feeds.

MATERIAL AND METHODS

The study was carried out on medicated feedstuffs, intermediate products medicated premixes and cleaning mixtures. In four years (2006 – 2009) 1454 samples were analysed. In 2006, 265 samples (207 of medicated feeds, 40 of medicated premixes and 18 of cleaning mixtures) were examined. In 2007, 454 samples (372 of medicated feeds, 59 of medicated premixes, 5 of intermediate products and 18 of cleaning mixtures) were investigated. In 2008, 382 samples (319 of medicated feedstuffs, 14 of medicated premixes, one intermediate product, 48 of cleaning mixtures) were examined. In 2009, 353 samples were analysed (292 of medicated feeds, 11 of medicated premixes and 50 of cleaning mixtures). The samples were taken from Polish feed manufactures by official veterinary inspectors or by manufacturers.

The official control of medicated feedstuffs includes the analysis of the following active substances: amoxicillin, chlortetracycline, doxycycline, lincomycin, tiamulin and tylosin. The agar diffusion method, known as the standard cylinder plate assay and agar well technique were applied for the determination of the antibiotics. The antibiotic activity was determined by measuring the diffusion of antibiotic in an agar medium inoculated with *Bacillus cereus* ATCC 11778 for tetracyclines and *Kocuria rhizophila ex. Micrococcus luteus* ATCC 9341 for the other antibiotics. Diffusion was shown by the formation of zones of inhibiotion in the presence of the microorganism. The sample was extracted using phosphate buffer pH 8 and methanol (for amoxicillin, lincomycin and tylosin), 1 N hydrochloric acid and methanol (tetracyclines) and 0,1 N sulphuric acid and acetone (for tiamulin). After shaking and centrifuging, the supernatant was diluted in the same buffer to obtain a theoretical concentration U_2 of 2 µg/ml. Diffusion through agar was carried out in plates, all four concentrations of the standard solution (S_2, S_1, $S_{0.5}$, $S_{0.25}$) and of the sample extract (U_2, U_1, $U_{0.5}$, $U_{0.25}$) being used in each plate (eight holes of 10 mm diameter were punched out of the agar medium). For one sample four plates were prepared. Applying solutions of each concentration four times the determination was subject to an evaluation of 32 zones of inhibition. The diameters of these zones were taken to be in direct proportion to the logarithm of the antibiotic concentration.

RESULTS AND DISCUSSION

A total of 1454 samples (1190 samples of medicated feedstuffs, 124 samples of medicated premixes, 6 samples of intermediate products and 134 samples of cleaning mixtures) were analyzed during the four-year study. The number and assortment of the analyzed samples are shown in Table 1 and Table 2.

Table 1. Number of samples of medicated feedstuffs, medicated premixes, intermediate products and cleaning mixtures and number of positive samples in 2006 - 2007.

Tablica 1. Broj uzoraka ljekovitih krmiva , ljekovitih premiksa, posrednih proizvoda i smjesa za čišćenje te broj pozitivnih uzoraka u 2006. i 2007. godini

	Number of samples Broj uzoraka		Number of non-compliant samples Broj neodgovarajućih uzoraka	
	2006	2007.	2006	2007
Medicated feedstuffs with amoxicillin	6	32	0	9
Medicated feedstuffs with chlortetracycline	23	169	0	8
Medicated feedstuffs with doxycycline	17	22	0	0
Medicated feedstuffs with lincomycin	29	9	0	0
Medicated feedstuffs with tiamulin	17	23	0	0
Medicated feedstuffs with tylosin	115	117	0	12
Total - Ukupno	207	372	0	29 (7,8%)
Medicated premixes	40	59	0	0
Intermediate products	0	5	0	0
Cleaning mixtures	18	18	6 (33,3%)	4 (22,2%)

Table 2. Number of samples of medicated feedstuffs, medicated premixes, intermediate products and cleaning mixtures and number of positive samples in 2008 - 2009.

Tablica 2. Broj uzoraka ljekovitih krmiva, ljekovitih premiksa, posrednih proizvoda i smjesa za čišćenje te broj pozitivnih uzoraka u 2008. i 2009.

	Number of samples Broj uzoraka		Number of non-compliant samples Broj neodgovarajućih uzoraka			
			2008 r.		2009 r.	
	2008 r.	2009 r.	C*	H*	C	H
Medicated feedstuffs with amoxicillin	50	29	8	0	8	0
Medicated feedstuffs with chlortetracycline	110	61	7	0	2	10
Medicated feedstuffs with doxycycline	6	2	5	0	0	0
Medicated feedstuffs with lincomycin	57	137	10	0	2	5
Medicated feedstuffs with tiamulin	37	10	0	5	0	0
Medicated feedstuffs with tylosin	59	53	0	25	0	0
Total - Ukupno	**319**	**292**	**30**	**30**	**12**	**15**
Medicated premixes	14	11	0		0	
Intermediate products	1	0	0		0	
Cleaning mixtures	48	50	5 (10,4%)		19 (38%)	

* C –an active substance content * H – homogeneity

In the majority of medicated feedstuffs, mainly lincomycin, chlortetracycline or tylosin occurred as active substances. Among the samples of medicated feedstuffs 116 (9,75%) were non-compliant with the declaration or they were not homogeneous – value of the CV was ≥ 15%. In all medicated premixes the antibiotic content was compatible with declaration. The analyzed samples of intermediate products with tylosin (2 g/kg) and chlortetracycline (1,5 g/kg) met requirements for the concentration and homogeneity. Among 134 samples of cleaning mixtures, 34 (25.37%) were positive. It means that they contained residues of an active substance used in medicated feedstuffs.

In Poland official control of medicated feedstuffs has been obligatory since 2006. These tests were carried out in order to check the quality of medicated feedstuffs and to ensure that the produced feedstuffs were homogeneous. The results showed that 9.75% of medicated feeds were non-compliant with the concentration of an active substance or they were not homogeneous. The possible reason for this may include failure to add the correct concentration, physical losses of the drug during the production process, loss by chemical degradation or the ineffectiveness of the extraction procedure (Lynas et al., 1998).

Among samples of cleaning mixtures 25,4% contained residues of an active substance used in medicated feedstuff. The probable reason was carry-over. Carry-over of drugs or additives from one medicated feed batch to the next non-medicated one during either manufacturing, transport or even at the farm can occur (Kan and Meijer, 2007; Kennedy et al., 1998; Lynas et al., 1998). Contamination of compound feeds is dependent on a number of factors including human error, production practice and handling procedures in the feed mill, during transport and on the farm. In feed mills, residual quantities of medicated feedstuff may be retained at various points along the production line, contaminating subsequent one as it is processed (McEvoy , 2002).

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SAŽETAK

Od 1. siječnja 2006. antibiotici, osim coccidiostatika i histomonostatika mogu se upotrebljavati samo u ljekovitim krmivima. Ljekovita krmiva su krmiva koja sadrže dozvoljeni ljekoviti premiks ili dozvoljeni posredni proizvod a služe za hranidbu životinja, bez dalje prerade. Zbog toga je potrebno dalje istraživanje da se prouči kakvoća ljekovitih premiksa i

krmiva. Uvjeti proizvodnje, oglašavanja i uporabe ljekovitih krmiva jasno su određeni. Sadržaj aktivne tvari u ljekovitom krmivu određuje se u opisu. Službena kontrola uključuje količinsko određivanje sadržaja aktivne tvari u 1g aktivne tvari i homogenost ovakvog krmiva. Kriterij za procjenu homogenosti je koeficijent varijacije (CV) $\leq$ 15%. Službena kontrola ljekovitih krmiva uključuje mikrobiološku analizu sadržaja sljedećih tvari: amoksilina, klortetraciklina, doksiciklina, linkomicina, tilozina i tramulina. Analizirani su: ljekovita krmiva, posredni proizvodi, ljekoviti premiksi i smjese za čišćenje. U četiri godine (2006 - 2009) ispitana su ukupno 1454 uzorka. Među ljekovitim krmivima 116 (9.75%) nije odgovaralo deklaraciji ili je homogenost bila netočna - vrijednost CV bila je $\leq$ 15%. Od 134 smjese za čišćenje 34 (25.34%) su bile pozitivne, što znači da su sadržavale taloge aktivne tvari upotrijebljene u ljekovitom krmivu.

Ključne riječi: aktivne tvari, ljekovita krmiva