Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 116, 113—119, 2009

UDC 636.2.053.084
DOI:10.2298/ZMSPN0916113A

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INFLUENCE OF PELLETING ON MICROBIOLOGICAL AND MYCOTOXICAL CORRECTNESS OF FEED MIXTURES WITH BENTONITE SUPPLEMENT

ABSTRACT: Influence of pelleting calf feed mixtures supplemented with bentoni on microbiological and mycotoxicological properties was investigated. Microbiological and mycotoxicological quality was investigated at the production day (day 0) and after 45 days of storage. Total count of microorganisms in the pelleted mixture, at the day 0 (280.000/g), was several times lower than in the powdered mixture (2.000.000/g). Similar results were obtained at day 45 when the total number of microorganisms in the pelleted mixture was 270.000/g and 1.800.000/g in the powdered mixture. Number of yeasts and molds at the production day in the pelleted mixture was 650/g, and in the powdered mixture it was 27.000/g. Similar results were obtained 45 days later when the number of yeasts and molds in the pelleted mixture was 540/g, and 16.000/g in the powdered mixture. There were 6 species identified in the pelleted mixture, and 9 species in the powdered mixture at the day of production. Similar mold species ratio in the pelleted (11) and powdered mixture (13) was found at day 45. In the examined samples representatives of *Fusarium* genus — *F. subglutinans* i *F. verticillioides* dominated. Number of sultite-redzcing clostridia in the mixtures, in both observed periods, was similar (below 1000/g of sample). By mycotoxicological analysis of mixtures at the production day, only trichotecene (T-2 toxin) presence was found in amount of 0,337 mg/kg. The applied technological procedure of pelleting with bentonite supplement, had positive influence on the improvement of microbiological and toxicological properties of mixture.

KEY WORDS: bentonite, calves, feed mixtures, micoorganisms, mycotoxins, pelleting

INTRODUCTION

Safe food production is an imperative for human food and animal feed, producers, today. Therefore, technological and technical procedures which contribute to the reduction of food contamination are becoming more and more important. In animal feed production, mixture pelleting is one of such proce-

dures. Positive effects of pelleting are: decrease of mixture decomposition, reduction of total number of microorganisms, increase of volume mass, decrease of dustiness, possible use of finely grinded feedstuffs, increase of manipulation possibilities (Đ o r đ e v i ć and D i n i ć, 2007; S r e t e n o v i ć Ljiljana at al., 1995). As a consequence of exposing the mixture to the influence of vapour, pressure and temperature, nutrients are being chemically transformed, and thereby digestibility of amilose, hemicellulose, cellulose and pentosan is increased (S t o j a n o v i ć, 2008, G r u b i ć et al., 1995). Due to increased temperature (between 70 i 80°C) some antinutritive ingredients of feedstuffs and mixtures decompose. Feed mixture pelleting has a positive effect on production results (daily gain, milk production), their better consumption and utilization. Pelleting presents a thermoplastic forming process when homogenized particles of powdered feed are pressed through die perforations in order to improve pellet quality (lasting and grinding durability). During feed mixture processing, different binding substances are used, among which Ca-lignosulphonate, Na and Ca-bentonite. Bentonite is a colloid clay of volcanic origin in form of hydratated aluminium-silicate composed of mineral montmorilonite (50—90%). Bentonite composition may vary, but most of the different bentonite types consist of replacable Na+, K+, Ca], Mg] ions, and according to the ions present they are named as sodium bentonite, potassium bentonite, calcium bentonite or magnesium bentonite. Bentonite has extremely large covering surface (1 g of bentonite covers the surface of 700—800 m^2). Chemical composition of bentonite varies depending on the deposition place and most often contains 46—58% SiO_2, 12—22% Al_2O_3, 0,20—0,40% K_2O, 0,04—0,08% Na_2O, 1,70—3,50% MgO, 3,30—5,90%, CaO, 3,50—4,70% Fe_2O_3. Burning loss ammounts to 12—17%. Due to amphotheric characteristics (accepts and releases hydrogen ions) it is used as supplement for rumen pH regulation in cattle (A d a m o v i ć et al., 2004; M u r r a y et al., 1990). Bentonite binds aflatoxins (B_1, B_2, G_1 i G_2) in fodder and decreases the presence of aflatoxine M_1 residues in milk (by 60 to 90%). However, its possibility to adsorb zearalenone and ochrataxin is limited (P a s h a i sar., 2008). Bentonite inclusion in cow rations contributed to the reduction of milk contamination with ^{137}Cs and ^{134}Cs from 50% to 80%. Bentonite adsorbs excesive NH_3 from rumen liquid when NH_3 concentration is high, and releases NH_3 when its concentration is low. This provides more efficient nitrogen utilization from ammonia for microbiological protein synthesis. Consequently, the resorption of NH_3 into blood, liver load and energy consumption for urea synthesis are decreased. Due to bentonite possibility to bind water, its volume increases as well as the digest volume in digestive tract. The enlargement of digest volume to the decrease of its passage speed through digestive organs, and thus provides longer activity of digestive enzymes and nutrien digestibility increase. Bentonite decreases Cu solubility in rumen and its content in liver, which can be useful for treating chronical Cu intoxications in animals. Disadvantage of bentonite, beside its affinity to bind certain minerals, is also an affinity to bind vitamines (H u w i g et al., 2001).

The goal of this investigation was to determine the influence of pelleting procedure of calves mixtures supplemented with bentonite on microbiological and mycotoxicological properties of mixtures.

MATERIAL AND METHODS

The investigated mixtures were produced in the Feed Mixture Industry Padinska Skela. Components were mixed with horizontal mixer (Buhler) with 3000 t capacity. Mixture pelleting was done using the press of the same manufacturer. Pellet diameter was 4 mm, and lenght 4 to 6 mm. Mixture composition is shown in Table 1. Bentonite used in the experiment was derived by a special technological procedure (impurity separation, drying, crushing and grinding) at the Institute for Technology of Nuclear and Other Raw Materials, Belgrade. Bentonite contained: 48,37% SiO_2; 22,39% $A1_2O_3$; 0,40% K_2O; 0,07% Na_2O; 1,81% MgO; 5,86% CaO; 4,73% Fe_2O_3; and 0,34% TiO_2. Size of particles was below 50 mm.

After feed mixture production, samples for microbiological and mycotoxicological analysis were taken (day 0). The mixture samples were kept in nylon bags during 45 days (period november-december), 20 cm above the floor, in ventilated, semi-dark and dry room. Average room temperature was 18°C.

Tab. 1 — Powdered and pelleted mixture composition, %

Component	% in mixture
Corn, ground	34,30
Barley, ground	10,00
Soybean, full fat	22,50
Sunflower meal, 33% UP	10,50
Wheat bran	15,00
Lucerna flour	3,00
Limestone	1,20
Dicalcium-phosphate	0,40
Salt	0,60
Vitamine and mineral premix	1,00
Bentonite	1,50
Total	100,00

Microbiological investigations were performed according to the *Regulations on maximal quantity of harmful materials and ingredients in fodder* (Sl. list SFRJ No. 2/90). Total count of bacteria, molds and yeasts as well as identification of pathogenic microorganisms (bacteria of fecal origin, *Salmonella* spp., sulfite reducing *Clostridium* spp.) was done in accordance to the method SFRJ No. 25/80.

Micotoxicological investigations. The presence of aflatoxin Bl (AFL Bl), ochratoxin A (OTA) and zearalenone (ZEA) was determined according to the standard method (Sl. list SFRJ No. 15/87), while diacetoxyscirpenol (DAS) and T-2 toxin were analyzed by applying the method of P e p e l j n j a k and B a b i ć (1991). Identification of potentially toxigenic fungi was done accor-

ding to Domsh et al. (1980) and Samson and van Reenen-Hoekstra (1988).

RESULTS AND DISCUSSION

Total count of microorganisms in the pelleted mixture at the production day (280.000/g) was several times lower than in the powdered mixture (2.000.000/g). Similar results were obtained at day 45 when the total number of microorganisms in the pelleted mixture was 270.000/g and 1.800.000/g in the powdered mixture. Number of yeasts and molds at the production day in the pelleted mixture was 650/g, and in the powdered mixture it was 27.000/g. Similar results were obtained 45 days later when number of yeasts and molds in the pelleted mixture was 540/g, and 16.000/g in the powdered mixture. There were 6 species identified in the pelleted mixture, and 9 species in the powdered mixture at the day of production. Similar mold species ratio in the pelleted (11) and powdered mixture (13) was found at the day 45. In the examined samples, representatives of *Fusarium* genus — *F. subglutinans* i *F. verticillioides* dominated. Number of sultite-reducing clostridia in the mixtures, in both measuring periods, was similar (below 1000/g per sample). Other pathogenic bacterial species were not determined (Table 2).

Tab. 2 — Microbiological properties of feed mixtures

Parameter	Powdered mixture		Pelleted mixture	
	Day 0	Day 45	Day 0	Day 45
Microorganism count/g	2.000.000	1.800.000	280.000	270.000
Yeast and mold count/g	27.000	16.000	650	540
Identified molds				
Absidia corymbifera	+	+		+
A cremonium fusidioides		+	+	
Acremonium sp.		+	+	+
Alternaria sp.		+	+	
Aspergillus flavus	+	+		+
Aspergillus fumigatus		+		+
Aspergillus niger	+	+	+	
Aspergillus versicolor	+			
Epicoccum purpurascens				+
Fusarium subglutinans	+	+	+	+
Fusarium verticillioides	+	+		+
Fusarium sp.	+		+	
Mucor sp.		+		+
Penicillium monoverticillata	+			
Penicillium sp.	+	+		
Rhizopus nigricans	+	+		+
Scopulariopsis brevicaulis		+		+
Pathogenic bacteria				
Salmonellae sp./50 g	0	0	0	0
Sulfite-reducing Clostridium/g	< 1000	< 1000	< 1000	< 1000
Coagulase positiv. Staph./50 g	0	0	0	0
Proteus sp./50 g	0	0	0	0
Escherichia coli/50 g	0	0	0	0

Among potentially toxigenic molds, it is important to emphasize the constant presence, of *A. flavus* (AFL B1) and *A. niger* (OTA) species in the basic powdered mixture (T j a m o s et al., 2004) as well as *Fusarium* spp. from section Liseola both at day 0 and day 45, *F. verticillioides* and *F. subglutinans*, potential moniliformine, beauvericine and fusiproliferine producers (L e v i ć, 2008), were also found in the pelleted mixture indicating viability of these molds under the pelleting conditions.

Inspite relatively great number of potential mycotoxin producers, only trechotecene (T-2 toxin) presence was determined in ammount of 0,337 mg/kg of mixture (Table 3) at the production day.

Tab. 3 — Presence of mycotoxins in feed mixtures

Parameter	Powdered mixture		Pelleted mixture	
	Day 0	Day 45	Day 0	Day 45
Aflatoxin B1	ND	ND	ND	ND
Zearalenone	ND	ND	ND	ND
Ochratoxin A	ND	ND	ND	ND
Trichotecenes (T-2)	0,337	ND	0,337	ND
Trichotecenes (DAS)	ND	ND	ND	ND

Legend: ND — not detected (< 0,0004 mg/kg AFLB1; < 0,037 ZEA; < 0,004 mg/kg OTA; < 0,04 DAS and T-2)

After 45 days of storage, mycotoxin presence was not detecte in the mixtures. This indicates that present mold species did not produce mycotoxins in quantities measurable by TLC detection methods under given conditions.

It can be concluded that the pelleting procedure of feed mixtures supplemented with bentonite at 1,5% level had positive effect on the improvement of microbiological and mycotoxicological properties of investigated mixtures.

ACKNOWLEDGEMENT

This paper was realized within TR 20016 project, granted by the Ministry of Science and Technological Development of the Republic of Serbia.

LITERATURE

A d a m o v i ć, M., L e m i ć, J., J o v i č i n, M., Magdalena T o m a š e v i ć - Č a n o v i ć, J o v i č i ć, M., Mira K o v a č e v i ć (2004): *Uticaj pufera na produkciju i sastav mleka i metabolički profil krava*, Biotehnologija u stočarstvu, 5—6, 195—202.

B o č a r o v - S t a n č i ć, Aleksandra, A d a m o v i ć, M., M i l j k o v i ć, A., Š t r b a c, S., S a l m a, N. (2008): *Mikotoksikološka kontaminacija hraniva i krmnih smeša i Banatu, AP Vojvodina*, Biotehnologija u stočarstvu, Vol. 24, 359—373.

Domsh, K. H., W. Gams, T.-H. Anderson (1980): *Compendium of Soil Fungi*, Academic Press, A Subsidiary of Harcourt Brace Jovanovich Publishers, London, England.

Đorđević, N., Dinić, B. (2007): *Hrana za životinje*. Cenzone-tech europe. Aranđelovac.

Grubić, G. (1995): *Neki fiziološki efekti peletiranja smeše koncentrata u ishrani teladi*, Savremena poljoprivreda, 43, 3, str. 119—123.

Huwig, A., Sreimund, S., Kappeli, O., Dutler, H. (2001): *Mycotoxin detoxication of animal feed by different adsorbents*, Toxicology Letters 122: 179—188.

Lević, J. (2008): *Vrste roda Fusarium u oblasti poljoprivrede, veterinarske i humane medicine* (eds. Institut za kukuruz "Zemun Polje" and Društvo genetičara Srbije), Cicero, Beograd.

Murray, P. J., Rowe, J. B., Aitchison, E. M. (1990): *The effect of bentonite on wool growth, liveweight change and rumen fermentation in sheep*, Australian Journal of Experimental Agriculture, 30 (1): 39—42.

Pasha, T. N., Mahmood, A., Khattak, F. M., Jabbar, M. A., Khan, A. D. (2008): *The effect of feed supplemented with different sodium bentonite treatments on broiler performance*, Turk. J. Vet. Anim. Sci., 32(4): 245—248.

Pepeljnjak, S., Babić, A. (1991): *Detection of trichothecenes mycotoxins, T-2, HT-2, DON and DAS by thin-layer chromatography and biological methods*, Prehrambeno-tehnol. biotehnol. Rev., 29, 65—70, Zagreb.

Sretenović, Liljana, Grubić, G., Adamović, M., Jovanović, R., Nada Đukić, Ranka Savićević, Bokić, N. (1995): *Uticaj peletiranja smeša koncentrata na zastupljenost nepoželjnih mikroorganizama i plesni*, VII Kongres mikrobiologa Jugoslavije. Zbornik rezimea, 183, Herceg Novi.

Stojanović, B., Grubić, G., Đorđević, N., Adamović, M., Radivojević, M. (2008): *Značaj peletiranja i korišćenja Na-bentonita u proizvodnji smeša za ishranu goveda*, Biotehnologija u stočarstvu, Vol. 24, 435—444.

Samson, R. A., van Reenen-Hoekstra, E.-S. (1988): *Introduction to Foodborn Fungi*, 3rd ed., Centraal bureau voor schimmelcultures, Baarn, Delft, Neetherland.

Sl. list SFRJ (1980): *Regulations on methods of microbiological analysis and superanalysis of foodstuffs. II Procedure for determining the presence, isolation and identification of microorganisms*, No. 25, 856—861, Beograd.

Sl. list SFRJ (1987): *Regulations on sampling methods and methods of physical, chemical and microbiological analysis of fodder*, No. 15, 422—449, Beograd.

Sl. list SFRJ (1990): *Regulations on maximal quantity of harmful substances and ingredients in fodder*, No. 2, paragraphs 8, 9 and 11, 29—30, Beograd.

Tjamos, S. E., Antoniou, P. P., Kazantzidou, A., Antonogoulos, D. E., Papageorgiou, I., Tjamos, E. C. (2004): *Aspergillus niger and Aspergillus carbonarius in Corinth raisin and wine producing vineyards in Greece: Population composition, ochratoxin A production and CHEMICAL CONTROL*, Journal of Phytopathology, 152 (4): 250—255.

УТИЦАЈ ПЕЛЕТИРАЊА НА МИКРОБИОЛОШКУ И МИКОТОКСИКОЛОШКУ ИСПРАВНОСТ КРМНИХ СМЕША СА ДОДАТКОМ БЕНТОНИТА

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Резиме

У огледу је испитиван утицај пелетирања крмних смеша за телад са додатком бентонита на микробиолошку и микотоксиколошку исправност смеша. Брашнаста и пелетирана крмна смеша за телад су произведене по истој рецептури. Микробиолошка и микотоксиколошка исправност смеша испитана је на дан производње (0-ти дан) и после 45 дана лагеровања. Укупан број микроорганизама у пелетираној смеши, на дан производње (280.000/g) био је вишеструко мањи од броја у брашнастој смеши (2.000.000/g). Слично је било 45 дана касније, када је укупан број микроорганизама у пелетираној смеши износио 270.000/g, односно 1.800.000/g у брашнастој смеши. Број квасаца и плесни на дан производње у пелетираној смеши био је 650/g, а у брашнастој 27.000/g. Слични резултати утврђени су 45 дана касније, када је број квасаца и плесни у пелетираној смеши износио 540/g, а у брашнастој 16.000/g. У пелетираној смеши на дан производње идентификовано је 6 врста, а у брашнастој 9 врста плесни. Сличан однос врста плесни у пелетираној (11) и брашнастој (13) утврђен је и 45 дана касније. У испитаним узорцима су доминирали представници рода *Fusarium — F. subglutinans* и *F. verticillioides*. Број сулфиторедукујућих клостридија у смешама, у оба термина контроле, био је сличан, односно испод 1000/g узорка. Остале врсте патогених бактерија нису идентификоване. Микотоксиколошком анализом смеша на дан производње утврђено је једино присуство трихотецена (Т-2 токсин) у количини од 0,337 mg/kg смеше. Примењени технолошки поступак пелетирања, уз додатак бентонита као везивног средства, имао је позитиван утицај на побољшање микробиолошке и токсиколошке исправности испитиваних крмних смеша.