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ЗА ПРИРОДНЕ НАУКЕ

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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 bentonite 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 sulfite-reducing 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 trichothecene (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, microorganisms, 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 (Đorđević and Dinić, 2007; Sretenović Ljiljana et 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 (Stojanović, 2008, Grubić 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 montmorillonite (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<sup>2</sup>). Chemical composition of bentonite varies depending on the deposition place and most often contains 46—58% SiO<sub>2</sub>, 12—22% Al<sub>2</sub>O<sub>3</sub>, 0,20—0,40% K<sub>2</sub>O, 0,04—0,08% Na<sub>2</sub>O, 1,70—3,50% MgO, 3,30—5,90%, CaO, 3,50—4,70% Fe<sub>2</sub>O<sub>3</sub>. Burning loss amounts to 12—17%. Due to amphoteric characteristics (accepts and releases hydrogen ions) it is used as supplement for rumen pH regulation in cattle (Adamović et al., 2004; Murray et al., 1990). Bentonite binds aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> i G<sub>2</sub>) in fodder and decreases the presence of aflatoxine M<sub>1</sub> residues in milk (by 60 to 90%). However, its possibility to adsorb zearalenone and ochratoxin is limited (Pasha i sar., 2008). Bentonite inclusion in cow rations contributed to the reduction of milk contamination with <sup>137</sup>Cs and <sup>134</sup>Cs from 50% to 80%. Bentonite adsorbs excessive NH<sub>3</sub> from rumen liquid when NH<sub>3</sub> concentration is high, and releases NH<sub>3</sub> when its concentration is low. This provides more efficient nitrogen utilization from ammonia for microbiological protein synthesis. Consequently, the resorption of NH<sub>3</sub> 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 chronic Cu intoxications in animals. Disadvantage of bentonite, beside its affinity to bind certain minerals, is also an affinity to bind vitamins (Huwig 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 length 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<sub>2</sub>; 22,39% Al<sub>2</sub>O<sub>3</sub>; 0,40% K<sub>2</sub>O; 0,07% Na<sub>2</sub>O; 1,81% MgO; 5,86% CaO; 4,73% Fe<sub>2</sub>O<sub>3</sub>; and 0,34% TiO<sub>2</sub>. 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 B1 (AFL B1), 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 sulfite-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
<b>Microorganism count/g</b>	2.000.000	1.800.000	280.000	270.000
<b>Yeast and mold count/g</b>	27.000	16.000	650	540
<b>Identified molds</b>				
<i>Absidia corymbifera</i>	+	+		+
<i>Acremonium fusidioides</i>		+	+	
<i>Acremonium</i> sp.		+	+	+
<i>Alternaria</i> sp.		+	+	
<i>Aspergillus flavus</i>	+	+		+
<i>Aspergillus fumigatus</i>		+		+
<i>Aspergillus niger</i>	+	+	+	
<i>Aspergillus versicolor</i>	+			
<i>Epicoccum purpurascens</i>				+
<i>Fusarium subglutinans</i>	+	+	+	+
<i>Fusarium verticillioides</i>	+	+		+
<i>Fusarium</i> sp.	+		+	
<i>Mucor</i> sp.		+		+
<i>Penicillium monoverticillata</i>	+			
<i>Penicillium</i> sp.	+	+		
<i>Rhizopus nigricans</i>	+	+		+
<i>Scopulariopsis brevicaulis</i>		+		+
<b>Pathogenic bacteria</b>				
<i>Salmonellae</i> sp./50 g	0	0	0	0
<i>Sulfite-reducing Clostridium</i> /g	< 1000	< 1000	< 1000	< 1000
<i>Coagulase positiv. Staph.</i> /50 g	0	0	0	0
<i>Proteus</i> sp./50 g	0	0	0	0
<i>Escherichia coli</i> /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 (Tjamos 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 (Lević, 2008), were also found in the pelleted mixture indicating viability of these molds under the pelleting conditions.

In spite relatively great number of potential mycotoxin producers, only trichotecene (T-2 toxin) presence was determined in amount 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 detected 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.

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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 лагеравања. Укупан број микроорганизама у пелетираној смеши, на дан производње (280.000/g) био је вишеструко мањи од броја у брашној 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 брашној смеши. Број квасаца и плесни на дан производње у пелетираној смеши био је 650/g, а у брашној 27.000/g. Слични резултати утврђени су 45 дана касније, када је број квасаца и плесни у пелетираној смеши износио 540/g, а у брашној 16.000/g. У пелетираној смеши на дан производње идентификовано је 6 врста, а у брашној 9 врста плесни. Сличан однос врста плесни у пелетираној (11) и брашној (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. Микотоксиколошким 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.