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EFFECT OF „BENTOPEL" ON THE QUALITY AND EFFICIENCY OF THE USAGE OF PELLETED FEED MIXTURES
UTICAJ „BENTOPELA" NA KVALITET I EFIKASNOST KORIŠĆENJA PELETIRANIH KRMNIH SMEŠA

Milan ADAMOVIĆ, Aleksandra BOČAROV-STANČIĆ **, Mihailo RADIVOJEVIĆ****
**Institute for Technology of Nuclear and other Mineral Raw Materials 11000 Beograd, Franchet d' Esperey Street 86, Serbia*
*** "Bio-ecological center" Ltd., 23000 Zrenjanin, P. Drapšina 15, Serbia*
****Institute PKB Agroekonomik, 11213 Padinska Skela, Zrenjaninski put bb., Serbia*
e-mail:m.adamovic@itnms.ac.rs

SUMMARY

This paper presents the results of the addition of the material for pelleting of feed mixtures ("Bentopel" - bentonite based additive) and its impact on physical and chemical characteristics, microbiological and mycotoxicological safety of pelleted feed mixtures for laying hens. Furthermore, the results on impact of pelleted feed mixtures with added "Bentopel" on growth, consumption, feed utilization and pH in blood serum and rumen fluid of calves was presented. The assessment of physical and chemical characteristics, microbiological and mycotoxicological safety of mixture for laying hens was conducted by comparing the experimental (E) and control mixtures (C). Inclusion of "Bentopel" in mixture E in the amount of 2% did not have significant impact on its chemical composition, with exception of silicium (1.12%:0.11%) and aluminum (0.21% : 0.02%) (E: C). The hardness of pellets in the experimental mixture was higher than in the control (6 Khal J/kg: 3.7 Khal J/kg), and the obliteration index was lower (10.7% :14.1%). The total number of bacteria in the mixture E was lower than in the mixture C (5,000/g:39,000/g). The total number of yeasts and molds in the mixture E was about three times lower (10/g : 30/g). The number of identified mold species was also lower in the mixture E than in the mixture C (3 : 8). Calves fed mixture supplemented with "Bentopel" (1.5%) had a higher average daily weight gain (1.084 kg:0.972 kg). The efficiency of the mixture utilization was better in E group of calves (1.74 kg:1.86 kg). PH values of the rumen content of calves at the age of 80 days in E and C groups were 6.54 and 6.28, while at the age of 120 days they were 6.39 and 6.14 respectively. PH values of blood serum at the age of 80 days were 7.45 and 7.40, while at the age of 120 days they were 7.49 and 7.40, in the E and C group respectively. In group E these values were closer to the optimal physiological values.

***Key words**: bentonite, feed mixtures, pelleting.*

REZIME

U radu su prikazani rezultati istraživanja uticaja sredstva za peletiranje krmnih smeša - "Bentopel" (na bazi bentonita) na fizičko-hemijske osobine, mikrobiološku i mikotoksikološku ispravnost peletirane krmne smeše za koke nosilje. Dat je i prikaz rezultata istraživanja o uticaju peletirane krmne smeše sa dodatkom "Bentopela" na prirast, konzumiranje, korišćenje hrane i pH u krvnom serumu i buragu teladi. Ocena fizičko-hemijskih osobina, mikrobiološke i mikotoksikološke ispravnosti smeše za koke nosilje obavljena je poređenjem ogledne-O i kontrolne-K smeše. Dodatak 2% "Bentopela" smeši O nije znatnije uticao na njen hemijski sastav, izuzev sadržaja silicijuma (1,12:0,11) i aluminijuma (0,21:0,02), (O:K). Tvrdoća peleta u smeši O bila je veća (6 Khal J/kg : 3,7 Khal J/kg), a indeks otiranja manji (10,7%:14,1%). Ukupan broj bakterija u smeši O je bio manji nego u smeši K (5.000/g : 39.000/g). Ukupan broj kvasaca i plesni u smeši O bio je tri puta manji (10/g: 30/g). Broj identifikovanih vrsta plesni je takođe bio manji u O smeši (3) nego u K smeši (8). Telad hranjena smešom sa dodatkom "Bentopela" (1,5%) ostvarila su veći dnevni prirast (1,084:0,972 kg). Efikasnost korišćenja smeše bila je povoljnija u O grupi teladi (1,74:186 kg). Vrednost pH sadržaja buraga teladi iznosila je 80. dana uzrasta 6,54:6,28, a 120. dana 6,39:6,14. Vrednost pH krvnog seruma 80. dana je iznosila 7,45:7,40, odnosno 120. dana 7,49:7,40 (O:K), i u O grupi je bila bliža optimalnim fiziološkim vrednostima.

***Ključne reči**: bentonit, krmne smeše, peletiranje.*

INTRODUCTION

Technological processes that contribute to the quality of feed are very important having in mind security as the main task of the manufacturers. One such procedure is the pelleting of feed mixtures. Pelleting is a process of passing of powdered feed through die in the presence or absence of water vapor. The effect of the use of heated water vapor and friction force is increased temperature of pellets (70-80 ^{o}C). This process leads to partial degradation of carbohydrates, and the result is increased digestibility of starch, hemicelluloses, cellulose and pentosanes. Pelleting also improves the taste and increases the metabolic energy. Physical form of pellets is similar to a natural form of grainy food which encourages eating and chewing and thus the secretion of saliva, which contributes to maintaining the pH of the rumen of ruminants. Pelleting allows more efficient use of finely grounded feeds and increases density of pellets. In addition there is the positive impact on reduction of decay, absorption of moisture and odors. Furthermore, decomposition and losses during transport are reduced. High temperature during pelleting leads to the reduction of total count of microorganisms *(Chukwuka et al., 2010; Lević and Sredanović, 2010; Đorđević and Dinić, 2007; Sretenović et al., 1995).* The additional result is a breakdown of certain anti-nutritional feed components (trypsin inhibitors, lectins, urease, peroxidase, lipoxygenase, myrosinase, glucosinolates, gossypol etc.) which are degradable at higher temperatures. Because of that the final result of pelleting is higher production, as well as better health of animals and economic effects of animal nutrition *(Grubic, 1995; Stojanovic et al., 2008).* Various supplements can be used in order to improve the quality (hardness) of pellets, such as: molasses, calcium-lignosulfonate, sodium or calcium-bentonite, a concentrate of cellulose fibers, and other substances of organic and inorganic origin *(Withlow and Hagler, 2009; Salari et al., 2009).*

Bentonites are white, light-weight rocks deposits composed mostly of salts of hydrated aluminsilicates of volcanic origin, consists of mineral montmorillonite (50-90%). It consists of exchangeable ions such as Na^{+}, K^{+}, Ca^{++}, Mg^{++}, etc. and depending on it, name of bentonite. The chemical composition of bentonite varies depending on the mining place. Usually it 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, and 3.50-4.70% Fe_2O_3. Bentonite has certain thermoplastic properties and because of that good results in the pelleting of feed mixtures can be achieved. Bentonite has a large binding capacity of liquids (water and oil). Layers of bentonite's crystal lattice are about 1 nm thick and are separated in presence of water molecules that enter the lattice (from 0.9 to 2.1 nm). In contact with water, bentonite increases the weight by 1.5 times and volume by 1.2 times. The Cation exchange capacity (CEC) of bentonite is 80-120 meq/100 g. Bentonite has a large covering surface (1 g covers of 700-800 m^2). Due to the amphoteric properties (receiving and releasing hydrogen ions) it is used also for preserving the rumen pH of cattle and consequently for the decrease of depressive effects of low rumen pH on fat content in milk. *(Adamović et al., 2009a; Kermanshahi et al. 2009; Pasha et al., 2008; Salari et al .2006).* Bentonite binds aflatoxins (B_1, B_2, G_1 and G_2) in feed as well as reduces the presence of residues of aflatoxin M_1 in milk (60 to 90%). Ability to adsorb zearalenone and ochratoxin is limited. Inclusion of bentonite in the diet of cows resulted in a reduction of milk contamination with ^{137}Cs and ^{134}Cs for 50% to 80% *(Stojanović et al., 2008).* Bentonite adsorbs NH_3 from the rumen, when its concentration is high, releasing it later, when its concentration is lower. This allows more efficient utilization of ammonia for protein synthesis by rumen microorganisms. In this way the absorption of NH_3 into blood is reduced and the conditions for liver activity are better as well as the amount of energy required for urea synthesis is lower. The volume of consummated feed in digestive organs can be increased as a result of bentonite ability to bind water. This further leads to the lower speed of food passage through the digestive organs, prolonged action of digestive enzymes, and thus the result is higher digestibility of nutrients *(Pasha et al 2008).* Bentonite can reduce the solubility of Cu, Zn and Mg in the rumen and can bind certain vitamins. So, this can become a problem when suboptimal quantities of these minerals and vitamins are present in the feed *(Adamović et al. 2009b).* On the other hand, reduction of Cu solubility may be one way for resolving the problem of chronic poisoning of animals with this heavy metal. The main reason for the use of bentonite in the process of pelleting of feed mixture is the increase of solidity and hardness of pellets. Besides that, pellets pass through the openings of matrix with less friction, so abrasion of equipment for pelleting is reduced.

The aim of this study, in which two of experiments were conducted, was to investigate the influence of “Bentopel” addition to pelleted feed. The objective of the first experiment was determination of physical and chemical parameters and hygienic status of complete feeds for laying hens. The objective of the second experiment was measuring of some important parameters of calf nutrition and physiology, such as weight gain, feed efficiency, and pH values of blood and rumen fluid.

MATERIAL AND METHOD
Experiment 1.

The material for pelleting of feed mixtures, based on natural bentonite (“Bentopel”) was produced by special technological process at the Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade. The chemical composition of “Bentopel” used in the experiments was determined AAS Analyst 300 and is shown in Table 1. Natural bentonite originated from the mine of Šipovo (Bosnia and Herzegovina). The relative portion of different granules in “Bentopel” is shown in Table 2.

Table 1. The chemical composition of “ Bentopel”

SiO_2 (%)	48.37	TiO_2 (%)	0.34
Al_2O (%)	22.39	Cd (ppm)	< 5
Fe_2O_3 (%)	4.3	Pb (ppm)	< 30
CaO (%)	5.6	Sb (ppm)	< 30
MgO (%)	0.81	Cu (ppm)	< 30
Na_2O %	0.07	Zn (ppm)	< 40
K_2O (%)	0.40	As (ppm)	< 20

Table 2. The relative portion of different granules in “Bentopel”

The class of size (μm)	M (%)	The class of size (μm)	M (%)
-63+61	12.60	-32+21	2.70
-61+46	2.70	-21+15	3.50
-46+32	3.50	-15+0	75.00

Complete feed mixture for laying hens, in pelleted form, with the addition of the “Bentopel" (2%) was produced in the livestock feed factory “Komponenta”, Ćuprija, Serbia. The diameter of pellets was 4 mm and the length was 4-6 mm. The composition of the mixture is shown in Table 3. The physical properties of pellets were determined by appropriate methods *(ASAE, 1996)* and criteria *(Thomas and Van der Poel, 2009).*

Microbiological investigations were performed according to the *Regulations on Maximal Quantity of Harmful Substances and Ingredients in Fodder (Off. Gazette of SRJ, No. 2/90).* The total count of bacteria, molds and yeasts as well as identification of pathogenic microorganisms (*E. coli,* coagul. positive *Staphylococcus* spp., *Proteus* spp., *Salmonella* spp., sulphito-reducing *Clostridium* spp.) were done according to *Regulations on methods of microbiological analysis and superanalysis of foodstuffs (Off. Gazette of SFRJ, No. 25/80).* The identifications of fungi were performed according to *Samson and van Reenen-Hoekstra, (1988).*

Table 3. Composition of feed mixture for laying hens,%

Component	Experimental mixture	Control mixture
Corn grain	45.9	45.9
Soybean meal, solvent extracted	12.5	12.5
Limestone	9.8	9.8
Extruded soybean	6.0	6.0
Sunflower meal, solvent extracted	9.30	9.3
Soybean meal, expeller extracted	5.0	5.0
Yeast	1.5	1.5
Wheat middling	4.0	6.0
Soybean oil	1.5	1.5
Na-bicarbonate	0.1	0.1
Mono-calcium phosphate	1.0	1.0
Salt	0.2	0.2
Premix	1.0	1.0
Methionine	0.1	0.1
Lysine	0.1	0.1
“Bentopel”	2.0	0
Total	100.0	100.0

Mycotoxicological investigations. The presence of aflatoxin B1 (AFL B1), ochratoxin A (OTA) and zearalenone (ZON) was determined according to Regulations on sampling methods and methods of physical, chemical and microbiological analysis of fodder (Off. Gazette of SFRJ, No. 15/87), while diacetoxyscirpenol (DAS) and T-2 toxin were analyzed by the method of Pepeljnjak and Babić (1991).

Experiment 2.

Complete feed mixture for calves (age of 1-4 months), in pelleted form, with the addition of the "Bentopel" (1,5%) was produced in the livestock feed factory "Inshra", Belgrade-Padinska Skela, Serbia. The diameter of pellets was 4 mm and the length 4-6 mm. The composition of the mixture is shown in Table 4.

Table 4. Composition of feed mixture for calves (%)

Component	Experimental mixture	Control mixture
Corn grain	34.3	34.3
Barley grain	10.0	10.0
Extruded full-fat soybean	22.5	22.5
Sunflower meal, solvent extracted, 33% CP	10.5	10.5
Wheat bran	15.0	16.5
Dehydrated alfalfa meal	3.0	3.0
Limestone	1.2	1.2
Di-calcium phosphate	0.4	0.4
Salt	0.6	0.6
Premix of vitamins and minerals	1.0	1.0
"Bentopel"	1.5	0
Total	100.0	100.0

Testing was carried out on two groups of Holstein calves. There were 10 calves in each group. The calves were kept in pens. The calves were engaged in the experiment from the age of 30 days until age of 122 days. The calves were fed with milk (the first 30 days of age) and milk replacer (from day 31 to day 80). Up to the age of 70 days, daily ration of liquid feed was 6 liters per calf. The meal was divided in two equal parts, 3 liters in the morning and the same amount in the evening. From 70 to 80 days of age, calves were fed with liquid feed once a day (in the morning) with the amount of 3 liters. The consumption of alfalfa hay as well as the mixtures of concentrated feed,, was *ad libitum*. The calves were supplied with water from the pressure-based watering containers.

RESULTS AND DISCUSSION

Experiment 1.

The results of the impact of "Bentopel" on the quality of complete feed mixture pellets for lying hens are shown in the Table 5.

Table 5. Quality of complete feed mixture pellets for laying hens

Parameter	Experimental mixture	Control mixture
Hardness of pellets(KhalJ/kg)	6.0	3.7
Obliteration of pellets (%)	10.7	14.1

The visual examination of pellets showed that in the experimental mixture their shape was regular and the surface was smooth, while the pellets of the control mixture were shorter and with somewhat damaged edges. Similar was noticed with the feed mixture for calves which included "Bentopel" in amount of 1.5%. The result of visual assessments of the pellets was in accordance with data about the hardness and obliteration. Hardness of pellets in the experimental mixture for lying hens was higher (6 Khal J/kg) in comparison with control mixture (3.7 Khal J/kg). The obliteration index of pellets was lower in experimental mixture (10.7%) than in control mixture (14.1%).

Due to the presence of similar ingredients, the chemical composition was very similar in both mixtures (Table 6) with exemption of Si and Al in the experimental mixture, which was the result of "Bentopel" addition (2%).

Table 6. Chemical composition of feed mixture for laying hens (%)

Parameter	Experimental mixture	Control mixture
Dry matter	90.63	90.41
Protein	17.56	17.78
Fat	5.20	5.32
Fiber	4.35	4.55
Ash	10.72	10.07
Si	1.12	0.11
Al	0.21	0.02
Ca	5.0	6.0
P	0.65	0.59

Total number of microorganisms (bacteria, yeasts and molds) 20 days after the production of mixtures in each of the two samples (Table 7) was much lower than the maximal allowed levels according to the *Regulations on maximal quantity of harmful substances and ingredients in fodder (Official Gazette of SRJ, No. 2/90).*

Table 7. Microbiological quality of feed mixtures for laying hens

Parameter	Experimental mixture	Control mixture
Total number of bacteria per gram	5,000	39,000
Total number of yeasts and molds per gram	10	30
Identified molds:		
Alternaria alternata		+
Aspergillus candidus		+
Aspergillus flavus		+
Aspergilus fumigatus	+	+
Chrysosporium merdarium		+
Fusarium verticillioides	+	+
Mucor mucedo	+	+
Rhizopus stolonifer		+

However, significantly lower total number of bacteria was noted in experimental mixture with addied Bentopel (5,000/g compared to 39,000/g in the control). Pathogenic species of bacteria (*E. coli*, coagulase positive *Staphylococcus* spp., *Proteus* spp., *Salmonella* spp. and sulfite-reducing *Clostridium* spp.) were not identified during present investigation. Difference in the total number of yeasts and molds in experimental and control mixture for lying hens was not statistically significant, unlike the number of identified mold species (Table 7). In experimental mixture only 3 fungal species were identified while in the control mixture the number of mycobiota was much higher. Detected species predominantly were saprobic field or storage fungi. Similar results about hygienic correctness of pelleted feed mixtures were obtained by *Adamović et al. (2009a), Bočarov-Stančić et al. (2010), Chukwuka et al. (2010) and Lević and Sredanović (2010) etc.* Mycotoxicological analysis did not establish the presence aflatoxin B1, zearalenone, ochratoxin A and type A trichothecenes (T-2 toxin and DAS). These results are not unexpected, considering that, with the exception of *A. flavus,* identified species of molds in the samples of control and experimental mixture, generally do not produce tested mycotoxins. The results of the microbiological safety and mycotoxicological correctness indicate a high level of control over the production process in the factory "Komponenta" where the mixtures were produced.

Experiment 2.

Chemical composition was very similar in both mixtures for calves (Table 8). The difference was in higher percentages of Si and Al in the experimental mixture, which was the result of the inclusion of "Bentopel" in the mixture (1.5%).

Table 8. Chemical composition of feed mixtures for calves (%)

Parameter	Experimental mixture	Control mixture
Dry matter	88.71	88.87
Protein	18.51	18.33
Fat	5.42	5.36
Fiber	7.80	7.72
Ash	3.96	5.13
Si	0.84	0.14
Al	0.16	0.02
Ca	0.72	0.74
P	0.60	0.58

The results of body weight, weight gain and efficiency of feed utilization are shown in Table 9. The average daily weight gain of calves in experimental group was higher for 112 g or 11.52%, as well as efficiency of feed utilization for 6.45% ($p<0.05$). Better weight gain and higher efficiency of feed utilization of chickens fed mixtures with addition of bentonite (1-2%), was also obtained in the study carried out by *Salari et al. (2006). Kermanshahi et al., (2009)* have obtained some similar results in their research focused on pelleted feed mixtures with addition of bentonite (0.5-1%). Due to the presence of aflatoxin B1 in those mixtures (500 and 1000 ppb) they have concluded that bentonite has the ability to absorb mentioned mycotoxin.

Table 9. Body weight, weight gain and efficiency of feed utilization

Parameter	Experimental mixture	Control mixture
Body weight at the beginning of experiment (kg)	54.05	54.55
Body weight at the end of experiment (kg)	146.20	147.90
Weight gain (kg/day)	1.084	0.972
Consumption of feed per one kg of weight gain (kg)	1.74	1.86

The pH values of rumen fluid and blood serum of calves are shown in Table 10. It can be noticed that on both day 80 and 120 these v *Microbiological investigations* alues were higher in the experimental group of calves. At the age of 80 days, the pH of rumen was 6.54: 6.8, and at the age of 120 days 6.39 : 6.14 (E:C). At the age of 80 days, the pH of blood serum was 7.45 : 7.0, and at the age of 120 days 7.49 : 7.40 (E:C), so it is obvious that those values were closer to optimal physiological values in experimental group (E).

Table 10. Values of pH of rumen fluid and blood serum of calves

Parameter	Experimental mixture	Control mixture
Day 80		
pH of rumen fluid	6.54	6.28
pH of blood serum	7.45	7.40
Day 120		
pH of rumen fluid	6.39	6.14
pH of blood serum	7.49	7.40

CONCLUSION

The quality of pelleted complete feed mixtures for laying hens supplemented with 2% of "Bentopel" was better than the quality of pellets without "Bentopel". Index of pellet obliteration was lower and the hardness was increased. The total number of bacteria, yeasts and molds was lower, as well as the number of identified mold species in experimental mixture. Feeding of calves with feed mixture with added "Bentopel" (1.5%) has led to the higher weight gain, improvement of feed utilization and increase of pH of rumen fluid and blood serum. When evaluating the suitability of a binding material for feed mixtures, some additional effects should be considered, such as capability of binding mycotoxins, radionuclides or ammonia, as well as the increase of consumed feed, maintaining of pH, prevention of growth of microorganisms, improved digestion of feed etc.

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