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INFLUENCE OF YEAST, PROBIOTICS AND ENZYMES IN RATIONS ON DAIRY COWS PERFORMANCES DURING TRANSITION **

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Abstract: The main goal of this paper was to investigate the influence of some additives included into high yielding dairy cows rations to overcome the problems in early lactation. These substances directly affect the productive performances of dairy cows as well as udder health.

The commercial name of investigated preparation is "**LIVEBIOS**" and it is composed of live yeast cultures selected from three strains *Saccharomyces cerevisisiae* in combination with probiotic bacteria and enzymes (*Lactobacillus casei, Streptococcus faecium, Aspergillus oryzae, Lactobacillus acidophilus*, 1,3-b and 1,6 D-Glucan, hemicellulase, Protease, Cellulase, Alpha amylase) which have the ability to modify the fermentation in rumen stimulating the development of ruminal bacteria and increasing the fibre digestion. Effects of this preparation have been investigated on 60 Holstein-Friesian cows divided into two groups. The diets were identical, and trial group received also 10 g of preparation Livebios daily. Application of Livebios started 15 days prior to calving and lasted until 60th day of lactation

As a research result improved in vitro disappearance of diet dry matter from roughage (alfalfa hay, maize silage and ensiled sugar beet pulp) was established.

Preparation Livebios influenced quantity and composition of the milk. The difference between trial and control group was 2.57 kg 4%FCM or 8.70% ($P<0.01$) and 7.16% milk fat ($P<0.05$).Trial group supplemented with Livebios showed lower somatic cells count by 7.3 percent points what indicated better health of cow's udder.

Based on the presented results it can be concluded that inclusion of preparation Livebios into diets for dairy cows in transition period can be recommended.

Key words: dairy cows, probiotics, yeast, transition period.

Introduction

The proper management strategies for dairy cattle are designed to prepare the cow for lactation and to minimize the incidence of metabolic diseases in the time of calving. High production of milk is followed by numerous problems generated during the dry period. In the recent time period extends from the last 3 weeks of gestation (close-up dry period) through the first 2 weeks of lactation (early fresh period) is called transition period. During this time the cows go from a low-maintenance phase to a high performance period in her productive life. Proper nutrition and management during the transition period is critical to successful lactation. The goal is to achieve peak milk production 5 to 6 weeks after calving, with a high peak yield and good continuing milk production.

Namely, primarily this relates to inadequate condition of heads of cattle which now can be corrected during this period. On the other hand, parturition is great stress to the animal, and period after parturition is characterized with great loss of appetite, animals enter in negative energy balance which can result in numerous health problems since high quantity of milk is not accompanied by adequate consumption of nutrients in the diet

Therefore, the genetic merit of the herd has increased considerably in recent years and this trend is continuing. High merit cows produce additional milk primarily as a result of differences in partitioning of nutrients between milk and daily live weight gain (*Gordon et al.*, 1995). Also recent evidence indicated that there is a negative relationship between milk yield and dairy cow fertility (*Pryce et al.,* 1998) with pregnancy rate to first service having decreased by approximately 1% per year over the last 10-20 years (*Mee et al.,* 1999). Previous studies have identified a strong relationship between the extent of negative energy balance (NEB) in early lactation and decreased conception rate (*Butler and Smith,* 1989).

In order to overcome these problems it is recommended to use some feed additives which are a group of feed ingredients that can cause a desired animal response in a non-nutrient role such as rumen pH shift, growth, or metabolic modifier. Four factors can be considered to determine if a feed additive should be used: anticipated response, economic return, available

research, and field responses (*Hutjens,* 1991). Response refers to expected performance changes the user could expect or anticipate when a feed additive is included. Several examples are listed below.

Higher milk yield (peak milk and/or milk persistency; increase in milk components (protein and/or fat); greater dry matter intake; stimulate rumen microbial synthesis of protein and/or volatile fatty acid (VFA) production; increase digestion in the digestive tract; stabilize rumen environment and pH; improve growth (gain and/or feed efficiency); minimize weight loss; reduce heat stress effects; improve health (such as less ketosis; reduce acidosis; or improve immune response.

The aim of this paper was to investigate the effects of application of the preparation LIVEBIOS which composed of live yeast cells selected of three *Saccharomyces cerevisisiae* strains with high fermentation capacity. Beside yeast cells this preparation contains probiotic bacteria and enzymes (*Lactobacillus casei*, *Streptococcus faecium*, *Aspergillus oryzae*, *Lactobacillus acidophilus*, β1,3-β1,6-D-Glucan, hemicellulase, Protease, Cellulase, Alpha amylase).

Yeast culture is a live culture of yeast and the media on which it was grown and dried so as to preserve the yeast's fermenting capacity. Several other types of yeast products are available from fermentation processes (such as brewers and distillers yeast). Yeast cells have been used for thousands of years. Their nutritive value is high; they are rich in enzymes, fatty acids, vitamin B complex, unknown growth factors and amino acids (more than 40% of total dry matter). Inclusion of yeast in diets for ruminants and non-ruminants leads to increase of consumption of dry matter, utilization of fibre and other nutritive substances and increase of daily gains. Yeast cells also absorb mycotoxins from food (*Bakutis et al.,2005)* and improve digestibility and absorption of minerals such as phosphorus, magnesium, calcium, copper, potassium, zinc and manganese (*Kinal et al., 2007).*

The word "probiotic" originates from Greek, and the mere translation of their meaning - *pro* = **for**, *biotic* = **life** -"for life" clearly denote in their effect. Probiotics are living organisms that are capable of colonizing the intestines after being ingested, with a positive effect on human and animal health. Mostly, these bacteria are bacteria of lactic fermentation (bifidobacteria, lactobacilles), are capable of stimulating the immunity mechanisms, producing antimicrobial substances and affect the metabolism in positive way - among others the production of vitamins, digestion process efficiency or lactose procession. Enzymes such as alfa amylase – degrade carbohydrates including starch into simple sugar; protease – degrades proteins into peptides and amino acids; celullaze – splits 1,4 glucose bonds in cellulose (fibres) and degrades it to

glucose. Addition of this preparation into diets for high yielding cows may expecte that all problems generated during the dry period would be overcome.

Material and methods

Effects of this preparation have been investigated on 60 Holstein-Friesian cows divided into two groups (control-C and trial-T). The experiment was performed in winter period at the farm of Holstein–Frisian Black and White dairy cattle.

Trial and control groups received identical diets, however trial group received also 10 g per head of preparation Livebios daily. Administration of preparation started during the stage of high gravidity (15 days before calving) and it lasted until 60th day of the lactation season.

Dry cows diet consisted of : 4.0 kg alfalfa hay, 20.0 kg whole maize plant silage and 5.0 kg concentrate mixture (18% total protein). Fresh cows diet consisted of: 4.0 kg alfalfa hay, 25 kg whole maize plant silage, 10.0 kg ensiled sugar beet pulp, 5.0 kg brewers grain, 1.5 kg soybean meal and 5.0 kg concentrate mixture (18% total protein) and they were balanced in all nutrition parameters. The main characteristics of Livebios according by guaranteed analysis is: crude protein min. 26.12%; crude fat min. 1.80%; crude fiber max. 11.0%; *Saccharomyces cerevisiae* min. 5,000 Billion Cells/g; *Lactobacillus acidophilus* and *Streptococcus faecium* min.10 Billion CFU/g.

Rumen fluid samples were collected 5h after the morning feeding with a stomach tube attached to a vacuum flask and pump from the three heads of cattle and filtered through the gauze Contents were placed in thermos bottles and immediately returned to the laboratory. The microbiology of rumen and its pH was investigated from rumen fluid and also in vitro dry matter disappearance of all used forage feeds of duplicate samples i.e. alfalfa hay, silage from the whole maize plant and ensiled sugar beet pulp, was determined with Tilley's and Terry's method (1963).

According to the method the feeds ground on 1mm diameter sieve have been put in the rumen fluid and incubated for 48 hours on 39°C. After that phase the sample was centrifuged, the liquid fraction was removed and then the incubation was done in the pepsin sour solution lasting 48 hours on 39°C. The undigested fraction of samples was separated with filter, and then dried in drier. The established difference in the mass of dry matter before and after incubation was put in relation to the mass of dry matter before incubation, and thus the digestibility of the dry matter was calculated.

The quantity of milk was recorded by Milkscope equipment and its composition was measured by Milkoscan apparatus. Somatic cell count was

recorded according microscoping method (*EN ISO 13366-1: 1997)*. The blood was withdrawn from jugular vein and the biochemical parameters (glucose, total billirubin, aspartate aminotransferase-AST, alanine aminotransferase-ALT, calcium and phosphorus) were investigated from blood serum at the beginning and end of the trial by automatic biochemical analyzer model Konelab 20. All data were statistically analyzed according to Snedecor and Cochran(1989).

Results and discussion

Dairy cows with a high milk production potential can increase dry matter intake (DMI) by as much as 250% between calving and the fourth week post-partum. In order to allow this increase it is important that cows remain on feed immediately prior to, and during calving.

However, published research has shown depressions in DM intake up to 40% beginning 7 to 10 days pre-partum.

The results in table 1. show disappearance of diet dry matter from roughage (alfalfa hay, maize silage and ensiled sugar beet pulp. It is quit clear that there is high statistical significant difference between control and trial group ($P<0.01$) for the alfalfa hay and only statistical significant difference ($P<0.05$) for silage from whole maize plant and ensiled sugar beet pulp for in vitro disappearance of dry matter. Based on these results all differences in animal performances obtained in this trial can be explained.

Table 1. *In vitro* dry matter digestibility of forage feeds influenced by preparation Livebios , %
Tabela 1. *In vitro* svarljivost suve materije iz krmnog hraniva pod uticajem preparata Livebios , %

Feed/Hranivo	Trials / Ogledi	
	C	T
Alfalfa hay/Seno lucerke	35.60^{A}	40.24^{B}
Whole maize plant silage/Silaža cele biljke kukuruza	64.44^{a}	66.12^{b}
Ensiled sugar beet pulp/Silirani rezanci šećerne repe	77.12^{a}	78.77^{b}

A,B significant at the level $P<0.01$/ A,B signifikantno na nivou $P<0.01$
a,b significant at the level $P<0.05$ / a,b signifikantno na nivou $P<0.05$

Results from this experiment are in accordance with those obtained by other authors. In a trial at Ohio State University, *Wang et al,* (1999) found that feeding a ration with 21% forage neutral detergent fiber and yeast culture positively affected dry matter intake, actual milk yield, 3.5% fat –corrected milk

yield and milk fat from 31 to 140 days in milk. The trial showed the importance of having optimal levels of nonstructural carbohydrates (NSC) in the diet. Cows in the treatment group with less than the optimal 37% to 38% NSC did not show the positive responses typically observed. Only cows receiving a properly formulated diet with proper energy levels fully realized the benefits.

Optimum rumen fermentation also drives the digestion rate. As the digestion rate increases, the rumen empties faster and cows can eat more feed. The animal's energy demand, the diet's energy density and physical fill all limit feed intake.

Cellulolytic bacteria activity accounts for the majority of fiber digestion in the rumen. These bacteria capture most of the energy from fiber when pH is maintained at or above pH 6.0. By nurturing a healthy, dynamic population of cellulolytic or fiber-digesting bacteria, **Livebios** culture helps increase fiber digestibility.

Very often cereal grains are routinely included in dairy rations to achieve high production levels, but, the rapidly fermented cereal grains provide substrates for rumen bacteria that produce lactate and decrease rumen pH. Fiber-degrading bacteria are inhibited as the pH drops below 6.0. Research suggests that adding yeast culture to the diet helps decrease lactate accumulation in the rumen by stimulating the bacteria that use lactate. Consequently, increasing lactate users in the rumen helps maintain rumen pH above 6.0 and increase fiber digestibility.

Three recent research trials performed by *Yoon et al.*(1998) showed that highlight rumen microbes improved ability to digest feedstuffs with yeast culture in the ration. The corn silage was inserted into the rumen of cannulated Jersey cows. The three corn silages differed in harvest maturity, moisture, processing, innoculation and cultivation practices. Corn silage digestion improved in the treatment group and remained consistent regardless of silage type. Treated cows showed increased dry matter, neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HC) disappearance.

Tab. 2 shows a great influence of preparation **Livebios** on quantity and composition of the milk. The difference between trial and control group was 2.57 kg 4%FCM (fat corrected milk) or 10.86%. Following records of the milk control the biggest difference was at the 1st one ($P<0.01$) and for the 2nd and 3rd were a little smaller but also statistically significant ($P<0.05$), indicating that inclusion this preparation into diets is the best way to overcome the postpartum problems.

The other researchers also were interested to investigate the effects of **Livebios** on milk production. In order to determine the effect of yeast culture on milk yield and milk composition in Chinese Holstein cows under Chinese management and feeding conditions the feeding trial was performed. The trial

conducted by *Wu Zilin* (1996) demonstrated that inclusion of yeast culture in the daily ration of Chinese Holstein lactating cows increased DMI 3.94%, milk yield 4.04, 3.5%FCM 7.07%, milk fat percent 5.77% and significantly increased the amount of milk produced, but had minor effect on percent of milk protein and lactose content. Yeast culture can also significantly increase body weights of dairy cows during the lactation period. Therefore, yeast culture is a relatively effective dairy feed additive under the Chinese feeding condition, and is worthwhile for general promotion for usage in China.

Table 2. Quantity and composition of milk influenced by preparation Livebios
Tabela 2. Količina i sastav mleka pod uticajem preparata Livebios

Parameter/Parametri	Trials / Ogledi	
	C	T
Average 4%FCM/ prosek 4%MKM	29.55A	32.12B
4%FCM (1st control)/ 4%MKM (1. kontrola)	24.33A	29.16B
4%FCM (2nd control)/ 4%MKM (2. kontrola)	30.81a	33.71b
4%FCM (3rd control)/ 4%MKM (3. kontrola)	30.00a	32.81b
Milk fat/Mlečna mast,%	3.91a	4.19b
Milk protein/Mlečni protein,%	3.05	3.11
Lactose/Laktoza,%	4.91	5.16
Fat free dry matter/Bezmasna suva materija,%	11.65	11.72

A,B significant at the level P<0.01/ A,B signifikantno na nivou P<0.01
a,b significant at the level P<0.05 / a,b signifikantno na nivou P<0.05

A summary of 7 yeast studies concluded cows feeding yeast supplements averaged 25.1 kg of 4% FCM compared to control cows at 23.5 kg (*Hutjens,* 1991). Early lactation cows had a significant increase in milk yield while mid lactation cows had no response (*Harris and Lobo*, 1988). Milk composition (fat and protein levels) response is also variable. Illinois (*Dann et al,* 2000) and Canadian workers (*Robinson and Garrett,* 1999) have reported significant increases in dry matter intake when yeast culture was fed to transition cows resulting in higher milk yields and less weight loss postpartum.

Schingoethe et al. (2004) used the yeast culture to evaluate its effect on production efficiency during the summer weather. Weekly daytime high temperatures during the 12 wk period averaged 33°C (28 to 39 °C). Yeast culture has improved mean daily 4% fat-corrected milk (31.2 and 32.0 kg/d), but percentages of milk fat (3.34 and 3.41) and true protein (2.85 and 2.87) were

similar for both diets. *Wu Zilin* (1996) pointed out that yeast culture increased the body weight of dairy cattle and the average daily gain (ADG) in the yeast culture group was significantly higher than in the control group over the trial period. The ADG for the control and yeast culture group was 0.163kg and 0.444kg ($P<0.02$) This has important implications for restoring the cow's body weight after calving and under the heat stress of summer months in maintaining milk production.

Robinson (1997) has shown that Holstein cows supplemented with a yeast culture preparation for approximately 14 days pre-partum and axactly 14 days post-partum lost less body condition pre-partum and was consistent with a higher weight gain. Milk and milk fat yield tended to be higher in cows fed yeast culture although milk protein production was not influenced.

The effect of **Livebios** on somatic cell count indicate that the trial group had smaller count of somatic cells (161.200) for 7.3 percent points comparing with control one (173.900) which point at better health status of **Livebios** cow′s udder. In both cases the number of somatic cell count was at satisfactory level having in mind farm′s conditions i.e. tied managing system.

In the beginning and in the end of trial the blood was taken from jugular vein and the most important biochemical parameters from blood serum were recorded. As it can be seen from table 3., all of them were in physiologically optimal limits which indicated stable health condition of heads and also that the stress occurring in this most productive physiological stage has been overcome. Physiologically optimal values were compared according by *Kaneko* (1989).

Table 3. Biochemical parameters of blood
Tabela 3. Biohemijski parametric krvi

Parameter/Parametar	Time of sampling/Vreme uzorkovanja				Phys. values of bl. par./ Fiz. vred. par. krvi
	Trial beginning/ Početak ogleda		Trial end/ Kraj ogleda		
	C	T	C	T	
Glucose/Glukoza, mmol/kg	3.43	3.42	3.27	3.53	2.5-4.2
Total billirubin/Ukupni bilirubin, mmol/l	2.62	2.23	3.80	2.73	0.2-8.5
Aspartate aminotransferase (AST), U/L	67.40	58.35	107.32	105.58	78-132
Alanine aminotransferase(ALT), U/L	23.57	21.50	32.20	33.50	14-38
Calcium/Kalcijum, mmol/l	2.60	2.62	2.35	2.32	2.4-3.1
Phosphorus/Fosfor, mol/l	2.07	2.25	2.07	2.08	1.8-2.1

Conclusion

Based on performed research and presented results it can be concluded that adding of preparation **Livebios** into diets for high yielding dairy cows in transition period was fully justified from the aspect of biochemistry and physiology of digestion in rumen, udder health condition, quantity and quality of milk. In general, all mentioned factors have great influence in increasing economical effects in cattle breeding.

Uticaj kvasaca, probiotika i enzima u obrocima na proizvodnost visokomlečnih krava u tranzicionom periodu**

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Rezime

Period zasušenja i rana laktacija karakterišu se brojnim problemima koji mogu da se uspešno premoste uključivanjem dodataka u hranu kao što su probiotici, kvasci, enzimi i dr.

U radu su ispitani efekti preparata yeasture koji predstavlja kompoziciju ovih dodataka a koji je uključen u obroke krava dve nedelje pre i osam nedelja posle telenja u količini od 10 g po grlu dnevno. Uključivanje pomenutih dodataka uticalo je na poboljšanje svarljivosti suve materije kabastih hraniva (sena lucerke, silaže cele biljke kukuruza i siliranog rezanca šećerne repe) što je konačno značajno uticalo na poboljšanje proizvodnih performansi odnosno povećanje količine mleka za 10.86%. Jednovremeno pomenuti dodaci povoljno su uticali na popravljanje zdravstvenog stanja vimena krava što se odrazilo na smanjenje broja somatskih ćelija za 7.3 procentnih poena. Najvažniji parametri krvi pre i posle eksperimenta kretali su se u fiziološki optimalnim granicama.

Navedeni argumenti su dovoljan razlog da se uključivanje pomenutih supstansi u obroke visokomlečnih krava u periodu zasušenja i rane laktacije preporuči u proizvodnji mleka.

Ključne reči: mlečne krave, probiotici, kvasci, enzimi, tranzicioni period

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