ISSN 1450-9156 UDC 636.085.2 DOI: 10.2298/BAH1103367P

BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN GROWING RABBITS FED DIFFERENT SOURCES OF CRUDE FIBER

M. Petkova, S. Grigorova, D. Abadjieva

Institute of Animal Science, Kostinbrod 2232, Bulgaria Corresponding author: m_petkova2002@abv.bg
Original scientific paper

Abstract: Crude fibers (CF) take a special place in the growing rabbit's nutrition. In depend on their origin and composition, CF have digestibility 17-25% in the roughages and up to 40-50% in the green and concentrate feeds. They are very important in the regulation of the processes of both digestibility and bacterial synthesis. The optimal CF quantity for growing rabbits is 12-15%. Alfalfa hay is preferred source of CF for rabbits, but it is too expensive. But, there are other sources of CF which are more suitable because of their price are lower. The aim of the present study was to investigate the effect of different sources of CF in the mixed rations for rabbits on blood biochemical changes, Volatile Fatty Acids (VFA) production in the ceacum and some fermentative parameters. In this respect we have interest to study the next sources of crude fibers: dry distillers' grain of wheat (DDGSw), produced by Bulgarian firm as a source except for crude fiber -13% and crude protein (CP) also -32%; meadow hay and as well as wheat straw as main and chipper sources of CF in the growing rabbits diets. Three compound feeds were prepared. All compound feeds contained 20% oats, 15% barley, 16.40% wheat bran, 10% soybean meal, 5% sunflower meal. The main sources of CF for the diets were: 30% Alfalfa hay (control group); 10% DDGSw + 20% meadow hay (Experimental group I, EG I) and 15% DDGSw + 15% wheat straw (Experimental group II, EG II). The obtained results showed that there were no any incidences of digestive disorders in all investigated groups. Improvement of cecal fermentation led to better NGR and FOM. We made conclusion that DDGSw in combine with meadow hay or wheat straw could use as alternative sources of CF for growing rabbits.

Key words: rabbits, crude fiber, DDGSw, caecum fermentation, fermentative parameters

Introduction

The importance of fiber in rabbit nutrition is well documented (De Blas et al., 1999; Gidenne, 2003; Chao and Li, 2008; Al-Dobaib, 2010). It is clarified and their optimal participation in daily ration for growing rabbits - 12-25% (Garcia et al., 2000). But the development of feed industry constantly offers new feed materials (Shurson, 2006). Information on their nutritional value is insufficient, and the effectiveness of different sources of fiber completely lacking experimental data. The traditional diets of growing rabbits rely entirely on the ground feedstuffs as alfalfa hay and dehydrated alfalfa. In the practice of rabbits nutrition recently dominated total mixed compound feeds in granulated state (Marinov, 2008). It is known that additional technological processes affect the nutritional value and the effective utilization. Application of DDGSw in the diet of rabbits is less studied. This new feed source is used with the success in Slovakia (Chrastinova et al., 2009), where as raw materials were used wheat, corn, barley, triticale, etc. In Bulgaria, for this purpose are mainly processed corn and wheat (www.allaboutfeed) and less barley. In our previous studies we indicate the inability of DDGSw to be used as the sole source of crude fiber in rations for rabbits and therefore the need to combine it with other sources of fiber. Interest in DDGSw is determined by the fact that it could used as the source of crude protein also. Notable are the advantages of DDGSw because of its low price. The testing of this product deserves attention because of the rapid expansion of ethanol production from grain. Very often when there is a little known feed component it is necessary to monitor the course of fermentative processes and to determine the fermentative parameters. The caecum is suitable for this purpose in experiments with rabbits. It was found that there was a positive correlation between the level of ADF in the diet and levels of acetic and butyric acid (Chao and Li, 2008).

The objective of our experiment was to determine the effects of DDGSw + meadow hay or straw as alternative sources of fibers on blood biochemical changes, caecum fermentation and some fermentative parameters in almost three-month-old White New Zealand bread rabbits.

Materials and Methods

Animals and diets. In the experimental station of Institute of Animal Science Kostinbrod was conducted a growth experiment with a total of 60 rabbits (New Zealand White) at the initial age of 45-50 days and average live weight of 1.3 kg. The animals were distributed into three groups - a control group and two experimental groups. The animals were raised in wire cages lined in a single layer and fed *ad libitum* with granulated TMR twice daily - at 8 and at 15h. Water was supplied via nipple watering trough. For more details see also *Grigorova et al.*

(2009). Three types of TMR for growing rabbis were formulated. Experimental design is shown on Table 1. All diets contained one and the same compound feed composed by: 20% oats, 15% barley, 16.40% wheat bran, 10% soybean meal, 5% sunflower meal, 1.50% calcium phosphate, 1% limestone, 0.40% salt, 0.50% Premix 6645, 0.05% cicostat, 0.10% DL-methionine, 0.05% lysine. The differences between the groups were on the kind of main sources of crude fibers for the whole diets: 30% alfalfa hay (control group), 20% meadow hay + 10%DDGSw (EG I); 15% wheat straw + 15% DDGSw (EG II). The TMRs were granulated with a pellets mill, die 4, pellets length 2 cm.

Table 1. Experimental design

Diet components, in %	Control group	Experimental group I	Experimental group II
		(EG I)	(EG II)
Compound Feed			
	70	70	70
Alfalfa hay	30	-	-
Meadow hay	-	20	-
Straw	-	-	15
DDGSw	-	10	15
TOTAL	100	100	100

Experimental procedures. The experiment lasted 42 days. Blood test has been made once a day at 8.00 PM, before feeding the animals. Blood samples have been taken from the ear vein of the experimental animals - 10 rabbits (5 male and 5 female) 3 times: on the 1st, 21st and 42nd days of the experiment. The following parameters have been studied: total protein, albumin, globulin, urea, cholesterol, triglycerides, and both AsAT and AlAT enzymes' activities. The same rabbits were slaughter on the day 42 and the samples from the caecum digesta were taken and were analyzed for: pH, total VFA, molar proportions of individual VFA and total amount of infusoria.

Chemical analyses, calculations and statistics. The total chemical composition of the feedstuffs was determined by the conventional Weende analysis. The pH post mortem was measured by portable pH-meter OP – 211 with a combined electrode penetrating 3 mm into the caecum content. All studied biochemical parameters were analyzed by Pentra 400 biochemical analyzer. Digesta was analyzed for total VFA by steam distillation by Marcham instrument. Molar proportions of individual VFA were determined after de-proteinization with mH₃PO₄ via gas-chromatograph Carlo-Erba HPGC 5300, series "Mega" with glass column on method of Kellogg (1969). Fermentative parameters (fermentative organic matter (FOM) according Demeyer and van Nevel (1975) and nonglucogenic ratio (NGR) after Orskov (1975)) were calculated based on the actual data of individual VFA. The total number of protozoa was determined by

microscope method with Fux-Rosenthal tally counter. The obtained results were statistically processed by Excel 2000, single factor, ANOVA program. Values are expressed as means \pm SD.

Results and Discussion

Composition of the experimental diets. The inclusion of 10 or 15% DDGSw in rations for growing rabbits in combine with meadow hay or straw does not cause drastic changes in chemical composition and nutritional value of the rations (Table 2). Obtained actual values and calculated parameters are very close in all three groups, which meet the objective of this study. In addition, it could say that our data on the composition of plant carbohydrates indicate that the test feed component DDGSw in combination with meadow hay (EG I) increased the content of NDF in the whole ration of 26.60%. The test feed component when was combine with straw (EG II) had the same increasing effect on NDF which were almost double (60.82 vs 39.17%) (*Petkova et al.*, 2011). The other major carbohydrate fraction – hemicellulose – was changed on similar manner. Its content had been increased from 23.03 to 29.45 and 43.96% for the three groups respectively.

Groups	Control group	EG I	EG II	Requirement**
DM, %	85.28	86.30	86.98	-
In % of DM				
CP	16.00	16.58	16.73	16.00
EE	2.65	2.91	2.78	2.0 - 3.0
CF	13.01	12.94	13.28	13 - 18
NFE	56.90	57.66	57.64	-
Ca, %	0.95	0.90	0.91	0.4 - 1.0
P, %	0.69	0.73	0.68	0.2 - 0.5
DE, kcal/kg*	2177	2280	2290	2100-2500

Table 2. Actual chemical composition and nutritive value of experimental TMRs

Blood hematological parameters. The influence of the experimental rations with DDGSw on the blood biochemical changes is shown by values of hematological parameters summarized in Table 3. Analysis of the age dynamics on the blood levels of total protein, albumin and globulin shows that these indicators were not affected by forage testing component. It should be noted that the all obtained values were within the reference values for this species (*Suckow and Douglas*, 1997). This applies to the three groups in both genders and in dynamics.

^{*} DE, Digestible energy (kcal/kg DM)= TDN x 4400 (NRC, 1985)

^{**} According Nutr. Requir. Rabbits, Second Rev. Ed., (1977)

The following average blood levels were obtained: total protein was 52.63 g/L (ranged 51.78 to 54.00 g/L); albumin was 29.23 g/L (ranged 27.38 to 30.35 g/L) and globulin was 23.46 g/L (ranged 22.48 to 24.74 g/L). The initial levels of urea in male rabbits were significant lower than those in female rabbits. The tendency to decrease of the levels of this parameter with growth and development of both male and female rabbits was obtained. Significant positive effect on the concentration of blood urea was observed in the two experimental groups of female rabbits. Experimental data for this indicator allows to summarizing; growth of animals lead to increase values. Maximum level 7.68 mmol/L was obtained in the group of female rabbits with the addition of 15% DDGSw and 15% straw in the experimental period of 42 days. The mean level for this indicator by all the controls was 6.38 mmol/L. Providing rations with DDGS we received significant decrease of blood cholesterol at the end of the experiment at the EG II independent of gender. Average cholesterol level was 0.96 mmol/L by all estimations and established values varied quite widely: from 0.43 to 1.40 mmol/L. The minimum and maximum values were established in one and the same EG II female rabbits. Similar to changes in cholesterol were the changes in the concentration of triglycerides in the blood. They ranged between 1.12 and 1.88 mmol/L, which determine the average value 1.46 mmol/L. In comparative terms, the values of this index are significantly higher in EG I in both gender. The growth had not statistically significant effect on the level of triglycerides. The activities of AlAT were within the range 19.02 and 26.47 U/L. Our values are similar to those established by Haris (1994) and Mircheva and Penchev (2005). Obtained results for AlAT activity convincingly demonstrate significant decreases in both treatment groups, regardless of gender. There was not observed in the dynamics of AlAT activity with increasing of age. In the studying the activity of other enzymes -AsAT - there were significant lower values in EG I. Maximum activity of 47.78 U/L was established in female rabbits in the reference period as a whole. The mean activity of all controls was 41.31U/L. The analysis of the results from all controlled hematological indicators warrant is assumed that tested combinations of DDGSw with meadow hay or straw causes significant changes in the concentrations of urea, cholesterol, triglycerides, and in the activities of AlAT and AsAT also. These changes can be applied to explain the metabolism of lipids in the body, the liver function and health status in general and level of productivity of rabbits.

Table 3. Blood biochemical parameters 1 , $x^* \pm SD$

Parameters	Male ♂			Female ♀			
	Control	EG I	EG II	Control	EG I	EG II	
			st day				
Total protein,	52.32	52.12	51.78	52.70	52.24	51.78	
g/L	1.10	0.75	0.79	0.87	0.69	0.62	
Albumin, g/L	29.20	27.38	29.30	28.24	29.16	29.18	
, _B =	0.21	1.08	1.36	0.25	1.35	0.55	
Globulin, g/L	23.08	24.74	22.48	24.46	23.08	22.60	
, <i>B</i>	1.22	1.51	0.82	0.95	1.20	0.15	
				h_1		h_1	
Urea, mmol/L	5.62	5.46	5.46	6.4	7.34	7.26	
,	0.11	0.12	0.21	0.35	0.50	0.45	
	F_1	A_2	A_3	F_1	A_2	A_3	
Cholesterol,	1.00	1.28	1.25	0.71	1.31	0.40	
mmol/L	0.01	0.03	0.01	0.06	0.06	0.05	
	n_1A_4	n_1	n_1D_1	n_1A_4	n_1	n_1D_1	
Triglycerides,	1.29	1.88	1.40	1.25	1.60	1.12	
mmol/L	0.01	0.24	0.02	0.01	0.28	0.09	
	$c_2a_3K_2$	c_2h_1	$a_3h_1D_2$	K_2	f_2	f_2D_2	
AlAT, U/L	26.00	19.02	23.32	26.02	19.04	23.30	
	0.77	0.79	1.42	1.13	1.18	1.07	
	a_4e_3	a_4k_3	e_3k_3	$n_7 h_3$	$n_7 c_2$	c_2h_3	
AsAT, U/L	46.50	31.98	42.82	45.24	31.68	46.22	
•	0.84	2.97	2.25	1.68	1.66	3.80	
	b_4	b_4		a_8	a_8d_7	d_7	
			On 2	1 st day			
Total protein,	52.38	52.43	52.90	52.73	52.48	53.13	
g/L	0.63	0.18	0.67	0.63	0.21	0.59	
Albumin, g/L	29.15	29.72	29.86	28.16	29.08	30.12	
	0.25	0.35	0.34	0.35	0.28	0.22	
	h_1E_1		h ₁	$e_1b_1E_1$	e_1k_1	b_1k_1	
Globulin, g/L	23.43	22.71	23.04	24.57	23.39	23.01	
	0.56	0.40	0.80	0.82	0.30	0.70	
Urea, mmol/L	5.25	5.68	5.88	6.50	7.52	7.56	
	0.08	0.09	0.09	0.10	0.48	0.15	
	$a_1d_1A_1$	d_1B_1	a_1B_3	$b_3e_2A_1$	e_2B_2	b_3B_3	
Cholesterol,	0.99	1.12	0.58	0.75	1.21	0.73	
mmol/L	0.01	0.05	0.06	0.40	0.05	0.05	
	$k_1b_2B_4$	k_1a_2	$a_2 b_2 H_1$	$n_2 B_4$	$n_2 b_4$	b_4H_1	
Triglycerides,	1.31	1.85	1.43	1.26	1.66	1.24	
mmol/L	0.04	0.05	0.11	0.02	0.09	0.06 a ₆	
	n_3	$n_3d_2C_2$	d_2	a_5	$a_5 a_6$		
AlAT, U/L	26.03	19.52	23.62	26.00	19.44	23.62	
	0.35	0.05	0.49	0.29	0.23	0.34	
	b_3	b_3	b ₃	b_6	b_6	b_6	
AsAT, U/L	46.74	32.12	43.28	45.47	32.09	44.76	
	0.34	0.27	0.38	0.18	0.44	0.20	
	a_6C_4	a_6	a_6C_3	$n_9k_3C_4$	n_9b_9	$b_9k_3C_3$	

	On 42 nd day							
Total protein,	52.36	52.67	53.77	52.77	52.75	54.00		
g/L	0.22	$0.29 e_1$	0.52	0.32	0.19	0.36		
	c_1		$c_1 e_1$	d_1		$d_1 c_1$		
Albumin, g/L	29.21	29.93	30.25	28.25	29.66	30.35		
	0.23	0.34	0.44	0.20	0.24	0.29		
	$e_2f_2C_1$	e_2	ef ₂	$a_1b_2C_1$	a_1f_1	b_2f_1		
Globulin, g/L	23.14	22.74	23.52	24.53	23.09	23.65		
	0.30 K_1	0.57	0.87	0.43	0.35	0.50		
				d_2K_1	d_2			
Urea, mmol/L	5.33	5.80	6.02	6.54	7.60	7.68		
	0.08	0.31	0.10	0.13	0.20	0.38		
	b_1N_1	N_2	b_1N_3	$a_2k_2N_1$	a_2N_2	k_2N_3		
Cholesterol,	1.02	1.04	0.57	0.78	1.20	0.43		
mmol/L	0.02	0.03	0.05	0.06	0.03	0.05		
	n_2N_4	d_2A_5	$d_2n_2E_2$	$n_3a_4N_4$	$n_3h_2A_5$	$h_2a_4E_2$		
Triglycerides,	1.34	1.65	1.50	1.30	1.74	1.38		
mmol/L	0.03	0.10	0.08	0.02	0.10	0.11		
	\mathbf{k}_2	k_2f_3	f_3	b_5	b_5d_5	\mathbf{d}_5		
AlAT, U/L	26.08	22.58	25.82	26.00	22.56	26.47		
	0.29	0.33	0.46	0.30	0.18	0.40		
	n_4	n_4a_5	a_5	a_7	$a_7 n_8$	n_8		
AsAT, U/L	46.76	34.69	45.10	45.39	35.04	47.78		
	0.34	0.47	0.49	0.22	1.17	0.30		
	$b_5c_3D_3$	b_5a_7	$c_3 a_7 N_5$	a_9D_3	a_9	a_9N_5		

^{*} every x is average from 5 estimations

$$\label{eq:Significance: A1...n, B1...n, B1..$$

Capital letters present comparison between genders, small letters – between groups.

Caecal fermentation. The date for pH, total VFA and protozoa level (Table 4) provide information for the rate of fermentation in the caecum. Male rabbits had greater pH than female in all groups. Rabbits from EG I were ranked the first in pH compared to the female groups and differ significantly (P<0.05) compared to the same female group and male EG II. Total VFA levels are presented with their absolute amounts. The gender has no effect on this parameter. Diets with DDGSw and meadow hay did not cause effect on TVFA. However, microbes in the rabbit's caecum produce more TVFA on DDGSw + straw based diets which may be linked with the efficiency of microbial growth rather than differences in total DM digestion by fermentation. The both Experimental groups II had statistically significant (P<0.05) higher levels of TVFA compared to their control groups. Concerning the protozoa level, the same tendency as TVFA was obtained.

Groups	рН		TVFA, mmol/l		Total amount of protozoa in 1 ml	
Female rabbits ♀						
Control group	6.99	0.18	23.75	1.97	37600	11.54
EG I	6.81	0.11 A	25.75	5.49	55200	14.27
EG II	6.97	0.11	31.90	5.31	73600	30.06
Male rabbits ♂						
Control group	7.06	0.11 a	23.75	3.54 a ₁	37600	2.19
EG I	7.43	0.29 aA	24.50	4.57 a ₁	59200	27.95
FG II	6 99	0.22	30.50	4.01	62400	25.76

Table 4. Rabbits' physiological parameters in caecum¹, $x^* \pm SD$

Capital letter present comparison between genders, small letters – between groups

The individual VFA production, their molar proportions and fermentative parameters calculated (Table 5) make quality characteristic of fermentation process in caecum. In general, acetate is the primary VFA produced by microbes, with more butyrate than propionate being formed. This supports the findings of other studied (*Chrastinova et al., 2009; Chrastinova et al., 2009a*). The following fermentation pattern (C₂:C₃:C₄) was obtained: 74: 8.5: 14. The molar percentage of acetate had a tendency to higher in the control diets than the both experimental groups. Diet with DDGSw + straw produced more acetate than DDGSw + meadow hay in both genders (73.60 and 74.04 vs 72.98 and 72.70%). Propionate production in female gender had small differences between three groups, but in the male gender EG I had significant higher (P<0.05) level than EG II.

Table 5. Qualitative and fermentative	parameters in caecum ¹ , $x^* \pm SD$
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Groups		ic acid		Propionic acid		c acid	SC***	FOM, kg/day	NGR
Female rabbits ♀		2		J	,				
Control group	75.84	1.51	8.25	0.91	12.03	0.83	3.87	8.97	10.57
EG I	72.98	1.13	8.24	1.15	16.19	1.56	2.60	9.40	11.28
EG II	73.60	1.10	8.49	1.37	13.89	2.03	4.02	9.13	10.34
Male rabbits ♂									
Control group	75.05	1.10	8.84	1.55	12.00	0.96	4.13	8.97	9.77
EG I	72.70	3.37	10.46	1.50 a	13.33	1.39	3.49	9.10	8.60
EG II	74.04	1.04	6,56	0.45 a	17.39	1.06	2.02	9.50	14.70

^{*} every x is average from 5 estimations

^{*} every x is average from 5 estimations

¹Significance: a, a_1 , A - P< 0.05

^{**} in mol/100mol TVFA

^{***} $SC = C_{4i} + C_{5i} + C_{5}$

¹Significance: a - P< 0.05

The production of butyrate was stimulated by the diet with DDGSw + straw in male rabbits, but in the other gender other experimental group had the maximum value. The other VFA production in the caecum – i-butyrate, i-valerate and valerate – is presented as sum (SC). In male rabbits feeding of both experimental alternative sources of fibers lowered values of SC. In the other gender, changes had indefinite character.

Calculated fermentative parameters. Feeding of alternative sources of fibers was characterized by better FOM in both genders. The comparison between genders stand out the better because of higher FOM in EG I in female group but in male rabbits FOM was stimulate from diet with DDGSw + straw. One more important fact concerning FOM was obtained: fully correspondence between the changes of FOM and butyrate production. Calculated NGR relations showed very similar values in female rabbits but with greater differences in male groups. The maximum level in female rabbits was calculated in diet wit DDGSw + meadow hay, but in male rabbits – in other experimental group. Used both fermentative parameters FOM and NGR had better values in experimental diets with alternative of alfalfa hay sources of fibers. Using of fermentative parameters shows intention the production of all individual VFA in caecal to be connected in one model. Whether because the amount of acetate in caecal is highest, but C_2 is believed to be the best indicator of changes in fermentation status in the caecum. It is known the presence of a negative depending on the utilization of metabolizable energy and the produced quantity of acetate. This means that rations with DDGSw and meadow hay or straw, where acetate has lower values than the control groups, provide better utilization of daily ration energy. Moreover, it was confirm by very good correspondence between the groups (control) with high acetate and the groups (experimental) with low FOM. The analysis of data from the fermentation in the caecum also shows very good agreement between the level of TVFA and FOM: the highest levels of TVFA in the two experimental groups correspond to the highest values of FOM.

Understanding the digestive physiology of the rabbit and how dietary components affect microbial growth efficiency in the caecum is a key to proper feeding management. Feeding strategy of rabbits combined with their digestive strategies could be much more of an advantage than when fed diets based on lignified forage, cereal grains and protein meals. The feeding strategy allows the animals to meet the dietary requirements for their high metabolic rate. Rabbits have high feed intake and fast feed transit time, which enable them to consume forage and meet nutritional requirements.

Conclusion

Providing diets with DDGSw in combination by meadow hay or straw for growing rabbits causes significant changes in the blood concentrations of urea, cholesterol, triglycerides, and in the activities of AlAT and AsAT also. These changes can be applied to explain the metabolism of lipids in the body, the liver function and health status in general and level of productivity of rabbits.

DDGSw with meadow hay or straw as sources of crude fiber alternative to alfalfa hay create better conditions for microbes` activity in the caecum and the result is obtained as a better fermentation and better fermentative parameters.

DDGSw with meadow hay or straw are successful alternative sources of fiber for growing rabbits.

Biohemijske i fiziološke promene kod zečeva hranjenih različitim izvorima sirove celuloze

M. Petkova, S. Grigorova, D. Abadjieva

Rezime

Sirova celuloza zauzima važno mesto u ishrani zečeva. U zavisnosti od porekla i sastava, sirova celuloza ima 17-25% svarljivosti u suvom materijalu i 40-50% u zelenoj masi i koncentrovanoj hrani. Ona je vrlo važna u regulaciji procesa svarljivosti i bakterijske sinteze. Optimalna koncentracija sirove celuloze za gajenje zečeva je 12-15%. Lucerkino seno je najbolji izvor sirove celuloze za zečeve, ali je mnogo skupo. Postoje drugi izvori koji su, zbog niže cene koštanja, mnogo povoljniji. Cilj istraživanja bio je da ispita efekte različitih izvora sirove celuloze na biohemijske promene u krvi zečeva, produkciju isparljivih masnih kiselina u cekumu i neke fermentativne parametre. U tom pogledu ispitivali smo sledeće izvore sirove celuloze: suva džibra pšenice kao izvora ne samo sirove celuloze od 13% već i sirovih proteina od 32%; livadsko seno kao i pšenična slama kao glavni i jeftiniji izvori sirove celuloze. Pripremljene su tri smeše za ishranu. Sve smeše sadrže 20% ovsa, 15% ječma, 16,40% pšeničnih mekinja, 10% sojine sačme i 5% suncokretove sačme. Glavni izvor CF u izhrani bilo je: 30% lucerkino seno (kontrolna grupa), 10% DDGSw + 20% livadsko seno (eksperimentalna grupa I, EG I) i 15% DDGSw + 15% pšenična slama (eksperimentalna grupa II, EG II). Dobijeni rezultati su pokazali da nije bilo nikakvih promena u digestivnom traktu kod svih ispitivanih grupa. Poboljšanje cekalne fermentacije poboljšalo je NGR i FOM. Zaključili smo da DDGSw u kombinaciji sa livadskim senom i pšeničnom slamom može da se koristi kao alternativni izvor sirove celuloze u gajenju zečeva.

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Received 30 June 2011; accepted for publication 15 August 2011