ФУРАЖИ И ХРАНЕНЕ

EFFECT OF OPTIGEN® ON SOME PARAMETERS OF RUMEN FERMENTATION IN YEARLING RAMS

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ВЛИЯНИЕ НА OPTIGEN® ВЪРХУ НЯКОИ ПОКАЗАТЕЛИ, ХАРАКТЕРИЗИРАЩИ ФЕРМЕНТАЦИОННИТЕ ПРОЦЕСИ В ТЪРБУХА НА ШИЛЕТА

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РЕЗЮМЕ

Проведен е физиологичен експеримент за установяване влиянието на добавката на Optigen® в дажбата върху ферментационните процеси в търбуха на шилета. Optigen® е източник на специфично протектиран небелтъчен азот (NPN) с индиректно контролирано (бавно) освобождаване в предстомашията. Създаден е специално за добавяне в дажбите на преживни животни. Използвани са шест броя шилета, кръстоски между породите Черноглава плевенска и Суфолк, с жива маса в началото на експеримента 45 ± 2 kg. Животните са изравнени по пол, жива маса и произход. Отглеждани са в закрито помещение, в индивидуални боксове, в експерименталната база на секция „Физиология“ към Аграрен факултет, Тракийски университет, Стара Загора. Опитът е проведен в два периода – контролен и опитен. По време на контролния период животните получават дажба, състояща се от 1,0 kg ливадно сено, 0,6 kg ечемичена ярма и 0,17 kg слънчогледов шрот. През опитния период към дажбата е добавен Optigen в доза 12 g на глава дневно. Проби от търбуховото съдържание са взимани в продължение на три дни за всеки експериментален период, 4-кратно, в рамките на деня (преди хранене, 1 ч, 2,5 ч и 5 ч – след хранене). Изследвани са следните показатели: концентрация на водородни йони (pH), концентрация на амоняк, общо количество на летливите мастни киселини (ЛМК), общ брой и родов състав на инфузории и целулозолитична активност в търбуха “in vivo”. Установено е, че добавянето на Optigen към дажбата на шилета увеличава и стабилизира стойностите на pH на търбуховото съдържание, които варират в тесни граници – от 6,61 до 6,78. Разликите с контролния период 1, 2,5 и 5 часа след хранене са доказани математически ($p < 0,001$, $p < 0,05$; $p < 0,01$). Optigen понижава общото количество на ЛМК и концентрацията на амоняк в търбуховото съдържание, доказано математически в часовете след хранене ($0,05 < p < 0,001$). Констатиран е инхибиращ ефект на Optigen върху популацията от търбухови инфузории ($p < 0,01$) успоредно с 1,3 пъти по-висока целулозолитична активност (9,02% срещу 6,98% през контролния период).

Ключови думи: Optigen®, ферментация в търбуха, храносмилане при овце, VFA

INTRODUCTION

Rumen metabolism is characterised with a relatively stable fermentation pattern, which is depicted through the following major parameters: total production of volatile fatty acids (VFA), molar VFA ratios, fermented organic matter to methane production ratio, level of anaerobic catabolic processes, size of ciliate and bacterial populations, amount of synthesised microbial protein (11). The protein metabolism in fore stomachs of ruminants consists of two primary interrelated processes - protein degradation by extracellular microbial enzymes and second second is microbial protein synthesis by rumen microflora during its life and development (10, 15, 16).The digestion in fore stomachs has always been a topic of special interest for researchers. Investigations on the ways of influencing protein degradation in rumen date back to the mid-20th century (7, 20). Nearly 50 years ago, Virtanen (33) established a very important fact, which would pose a new direction in rumen digestion research – that ruminants were able to convert non-protein nitrogen in milk protein. In Bulgaria, several experiments were performed to evaluate the effect of non-protein nitrogen compounds under a different form with regard to their slower hydrolysis (27, 31, 32). During the last years, the interest towards the utilisation of the so-called protected proteins in ruminant diets has increased. The purpose of their application is to prevent the complete degradation of proteins in the rumen and thus, to decreased rumen ammonia concentrations. At the same time, protected proteins provide an adequate amount of nitrogen to rumen microflora for microbial protein synthesis (1, 4, 15) A number of commercial products containing protected proteins are available on the global markets. Such a product is Optigen® – manufactured by Alltech, USA. According to the producer, Optigen is a source of specifically protected non-protein nitrogen (NPN) with controlled (slow) release in the fore stomachs of ruminants. It improves feed conversion and has a beneficial effect on the productivity and health of animals, optimises the costs of formulating ruminant diets (1, 4, 16, 29).

The available literature indicates that on a global scale, the researchers attention has been focused on the effect of the preparation on production traits, milk yield and composition (1, 2, 4, 8, 22, 23) or ruminal fermentation characteristics of cattle (12, 29) and beef cattle (21). So far, there is no similar study related to the effect of Optigen on the physiology of fore stomachs in small ruminants in Bulgaria.

That is why, the present study's aim was to anwer the question whether the replacement of a part of dietary protein with Optigen (at a dose recommended by the manufacturer) would alter the main parameters of rumen fermentation in small ruminants.

This is a pilot study at a national scale, part of an broader experiment designed to evaluate the effect of Optigen on rumen fermentation, duodenal chyme content, peripheral blood parameters and ethological indices in yearling sheep. All data from the research are to be published in near future.

MATERIAL AND METHODS

The experiment was conducted with six yearling rams, Pleven Blackhead × Suffolk crosses. The animals were housed indoor, in individual boxes at the Experimental Base of Animal Physiology Unit to the Faculty of Agriculture, Trakia University – Stara Zagora. The average live body weight of animals at the beginning of the experiment was 45±2 kg. The gender, body weight and origin of animals were uniform.

The experiment comprised two periods: control and experimental. The latter started after a 10-day pause necessary for adaptation of experimental animals to the new ration. The duration of the adaptation period was compliant to the physiology of experimental animals. In previous studies of ours, this duration had no negative effect on the reliability of our results. The rearing, feeding and microclimatic conditions during the experimental period were identical to those during the control period. Prior to the trial, the animals were fitted with cannulae of the dorsal rumen sac according to Aliev (3). A 2-week post

operation period was allowed, and consequently, the animals were fed a ration with composition and feed constituents as shown in Table 1. The ration was composed in a way such that protein balance in the rumen of experimental animals was ≥0, here – 0.93 g. In this study, only the effect of Optigen® on rumen parameters were monitored. The daily and total weight gain during both periods were not controlled.

During the experimental period, the ration was supplemented with Optigen at a daily dose of 12 g per animal. To obtain a balanced ration, the sunflower meal was excluded and barley mesh amount – increased by 100 g. The dose was selected following the recommendations of the manufacturer Alltech. According to the company specialists, the recommended daily dose of the preparation for small ruminants is 10-20 g. This dose was conforming to safe feeding guidelines and did not exceed the recommended daily dose of 0.3 g urea per kg body weight. After the addition of 12 g Optigen during the experimental period, the rumen protein balance changed insignificantly remaining within the reference limits – 0.6 g. During that period, the sunflower meal was totally excluded from the diet and replaced with Optigen, to realise the aim of the study – evaluation of the effect of plant protein replacement with synthetic protein. In order to preserve the rumen protein balance during the experimental period, the barley mesh amount was increased by 100 g. The dietary nitrogen levels during the control and experimental periods were 33.17 g and 32.26 g respectively.

The origin of non-protein nitrogen in Optigen is not specified, it is a trade secret of the manufacturer.

The ration was offered twice daily – at 8.00 AM after collection of first set of samples, and at 1.00 PM. Feed consumption was recorded at a daily basis.

After a 10-day adaptation period to the new ration, rumen content was sampled for 3 consecutive days, 4 times a day: before feeding, 1h, 2.5 h and 5 h after feeding with a 100-ml pipette, introduced at a depth of 15 cm through the cannula.

During the experimental period, the ration of rams was supplemented with the product Optigen, produced by Alltech, at a daily dose of 12 g per animal. The preparation was mixed with the concentrate.

The following rumen content parameters were investigated:

- Hydrogen ion concentration (pH),
- Ammonia concentration,
- Total volatile fatty acid (VFA) concentration,
- Total counts and generic composition of rumen ciliates,
- In vivo cellulolytic activity in the rumen

Routine methods of analysis, as described in previous studies of ours (24, 28) were used. Statistical analysis was done with Statistica 6.0

Table 1. Chemical composition of the ration for yearling rams with body weight of 45 kg

Feed	Control period							Experimental period						
	Kg*	DM, kg	FUG	PDI, g	BPR, g	Ca, g	P, g	Kg**	DM, kg	FUG	PDI, g	BPR, g	Ca, g	P, g
		1.61	1.54	90	≥0	6.8	3.5		1.61	1.54	90	≥0	6.8	3.5
Meadow hay	1	0.87	0.6	64	-3	6.53	2.26	1	0.87	0.6	64	-3	6.53	2.26
Barley mash	0.6	0.52	0.8	57	-18	0.31	2.03	0.7	0.61	0.94	66.5	-21	0.36	2.37
*Sunflower meal	0.17	0.15	0.14	19.89	21.93	0.70	1.84							
**Optigen								0.012				24.6		
Daily intake		1.54	1.54	140.89	0.93	7.54	6.13		1.48	1.54	130.5	0.6	6.89	4.63

DM – Dry matter, FUG – Feed Units for Growth, PDI – Protein truly digestible in small intestine, BPR – Balance of protein in the rumen.

(Windows) software and ANOVA test. Variables are presented as mean values ± standard deviation (SD). For comparison of different parameters the one way ANOVA test was used.

RESULTS

The hydrogen ion concentration-pH is essential for rumen fermentation. As seen from Table 3, pH ranged within the normal range – from 5.82 to 6.57 during the control period and 6.50 – 6.87 during the experimental one. It is well acknowledged that standard feeding results in rumen fluid pH between 5.4 – 7.4, as well as that the narrow range of pH values was largely due to the substantial buffering capacity of rumen bicarbonates and phosphates. This effect is attributed to the continuous flow of ingested saliva and the constant absorption of VFA through the ruminal wall, ion absorption and exchange etc. The addition of Optigen results in narrower range of pH variation. We have also observed that by the 1st post feeding hour, rumen pH remained almost unchanged unlike the control period, when pH value was statistically significantly lower ($p < 0.001$). In the subsequent periods (2.5 h and 5 h) the trend was restoration of pre-feeding values. In all sampling periods, pH attained higher values after Optigen supplementation, which were statistically significant in post feeding hours (Table 2).

The rumen VFA concentrations (Table 2) showed unidirectional tendencies during the control and experimental periods – increase after feeding and similar values before feeding – about 58 mmol/l. The effect of Optigen supplementation was very obvious – statistically significantly lower total VFA levels by about 15 mmol/l after feeding ($0.01 < p < 0.001$).

The total counts of rumen ciliates is shown in Table 3. During the control period, the ciliate population size decreased by the 1st post feeding hour ($p<0.001$), most probably as a results of the reduced rumen pH and fermentation processes. Ciliate counts increased substantially 2.5 and 5 hours after feeding ($p < 0.05$; $p < 0.001$). A similar tendency was established during the experi-

Table 2. Rumen parameters

Period	Time of study								
	before feeding			1 h after feeding		2,5 h after feeding		5 h after feeding	
	n	x	$\pm S_x$	x	$\pm S_x$	x	$\pm S_x$	x	$\pm S_x$
Hydrogen ion concentration (pH)									
Control	12	6.57	0.09	5,82 aaa	0.08	5.97	0.26	6,27 c eee	0.07
Experimental	12	6.78	0.07	6,85***	0.06	6,61*d	0.08	6,5 c ee	0.09
Ammonia concentration (mg/100 ml)									
Control	12	10.14	0.79	24,7 aaa	0.76	16,80bbbddd	1.83	11,75 eee	1.59
Experimental	12	8.76	0.7	22,73 aaa	1.69	14,55 bbbdd	1.61	9,36 eee f	1.38
Total volatile fatty acid (mmol/l)									
Control	12	57.08	2.19	83,33 aaa	4.9	83,96 bbb	2.95	82,92 ccc	3.11
Experimental	12	58.44	3.73	65,58**	3.02	67,19***b	2.26	65,73***	2.3

** - comparison of results between control and experimentally group; a - comparison of results before feeding and 1 h after feeding*
b - comparison of results before feeding and 2.5 h after feeding; c - comparison of results before feeding and 5 h after feeding
d - comparison of results 1 h and 2.5 h after feeding; e - comparison of results 1 h and 5 h after feeding
f - comparison of results 2.5 h and 5 h after feeding

mental period as well. The addition of Optigen resulted in obvious reduction of the total rumen ciliates counts with considerable differences ($p < 0.01$) 2.5 and 5 hours after the morning feeding.

The supplementation of experimental rations with Optigen had an insignificant effect on generic composition of rumen ciliates (Table3). The Epidinium genus population has increased ($p < 0.001$), the Ophryoscolex genus counts were significantly reduced ($p < 0.001$) and the other studied genera did not changed considerably.

DISCUSSION

The main incentive of the present experiment was to evaluate whether the effect of the preparation for large ruminants, as affirmed by the manufacturer (Alltech-USA), was also true for small ruminants, as well as to investigate the mechanism of its biological effect in order to optimise dietary protein costs for ruminant rations

The results presented in this article are a part of a large-scale study on the effect of Optigen®

Table 3. Total counts and generic composition of rumen ciliates

Period	Time of study								
	before feeding			1 h after feeding		2,5 h after feeding		5 h after feeding	
	n	x	$\pm S_x$	x	$\pm S_x$	x	$\pm S_x$	x	$\pm S_x$
Total counts of rumen ciliates ($10^3/cm^3$)									
Control	12	211.46	23.32	99.33aaa	2.49	155.21b ddd	13.21	219.42f	0.07
Experimental	12	152.13	30.68	84.17	13.33	95,54**	21.24	110,58**	21.97
Entodinium sp.									
Control	12	93.38	1.47	90.79	1.11	92.25	1.62	92.00	22647
Experimental	12	92.38	1.86	92.88	1.67	90.54	2.28	87.04	46813
Diplodinium sp.									
Control	12	1.54	0.39	2.58	0.46	2.33	0.53	1.71	45.00
Experimental	12	2.17	0.43	1.83	0.59	3.58	1.1	3.29	1.07
Epidinium sp.									
Control	12	2.00	0.90	0.00 a	0.00	2.04 dd	0.64	2.29	0.89
Experimental	12	2.96	0.94	1.38 ***	0.33	1.58	0.46	3.92	1.1
Ophryoscolex sp.									
Control	12	0.00	0.00	2.54 aaa	0.49	0.00 ddd	0.00	0.00	0.00
Experimental	12	0.00	0.00	0.00 ***	0.00	0.00	0.00	0.00	0.00
Isotricha sp.									
Control	12	3.08	0.62	4.13	0.57	3.38	0.61	4.00	0.80
Experimental	12	2.5	0.74	3.92	1.13	4.29	1.01	5.75	1.57
Dasytricha sp.									
Control	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Experimental	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**comparison of results between control and experimentally group; a - comparison of results before feeding and 1 h after feeding*

b - comparison of results before feeding and 2.5 h after feeding; d - comparison of results 1 h and 2.5 h after feeding

*f - comparison of results 2.5 h and 5 h after feeding; a, b, f – $p < 0.05$; **, dd – $p < 0.01$; ***, aaa, ddd – $p < 0.001$*

on rumen fermentation, the size and metabolic activity of rumen ciliate populations, the concentrations of some rumen fluid and duodenal content metabolites in yearling rams.

The rumen pH influences at a considerable extent proteolytic activity (5). Rumen proteolytic activity varies according to the type of ration (26). According to Jagos (17), when rumen pH is about 6.5, the ammonia concentrations are optimal from point of view of fermentation. High pH values are beneficial for the development of stimulating for metabolic activity of rumen bacterial colonies, which reflects on the extent of dietary dry matter degradation. Rumen pH is directly depending on the molar ratio of volatile fatty acids.

Volatile fatty acids are a product of carbohydrate degradation in the rumen of ruminants. A small amount is obtained from residues of amino acids deamination after feeding ruminants high-protein rations (20). Numerous factors influence the VFA concentrations in ruminants' fore stomachs. Limiting factors are the diet composition, frequency of feeding, inclusion of various nutritional supplements etc. The so-called endogenous factors as rumen pH, ammonia concentration, metabolic activity of rumen microflora and its population size also influence rumen VFA levels (30). The issue about rumen VFA concentrations is still disputable and should be interpreted in an integral manner depending on several limiting factors. In our view, the low VFA level demonstrated during the experimental period of the trial could be attributed to increased absorption through the rumen wall and particularly, to increased evacuation to the more distal alimentary tract compartments. After the supplementation of the ration with Optigen we observed increased VFA concentrations in the duodenal chyme (unpublished data). Lower VFA rumen concentrations are believed to be the direct cause for increased pH after Optigen supplementation.

Ammonia is a primary metabolite of proteins and non-protein nitrogen compounds in the rumen, resulting from the activity of rumen microflora. Each type of feed is characterised by a specific level and dynamics of ammonia genesis. Rumen ammonia as a parameter is directly limited by dietary easily degradable proteins.

In our studies, the dynamics in ammonia levels was the same in both periods. From about 10 mg/100 ml before feeding, a peak occurred on hour 1 with almost twice higher levels, followed by gradual decrease to values, comparable to initial ones by hour 5. Similarly to the Optigen effect observed for rumen VFA, the supplement induced lower rumen ammonia concentrations by all studied time intervals, despite the lack of statistically significant differences. The results showed that the effect of Optigen on ammonia concentrations was comparable to the anticipated one, declared by the manufacturer.

Regardless of the lack of statistically significant differences in *in vivo* rumen cellulolytic activity (Fig. 1) it could be affirmed that Optigen supplementation had a definite effect – the cellulolytic activity was 1.3 higher during the experimental period as compared to the control one.

This fact is very important from practical point of view, as the degradation of crude fibre

Period	*cellulolytic activity in the rumen (%/24 h)*		
	n	*x*	$\pm S_x$
Control period	*12*	*6.98*	*2.05*
Experimental period	*12*	*9.02*	*1.03*

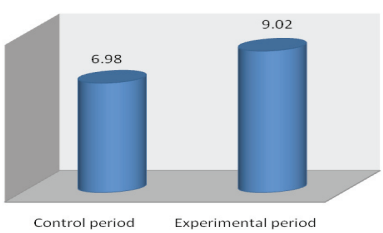

Fig. 1. Cellulolytic activity in the rumen a yearling rams

makes the content of plant cells, building their walls, available for utilisation. It is hypothesised that the mechanism of cellulose degradation in the fore stomachs of ruminants consists in production of extracellular cellulose by cellulolytic bacteria. Rumen holotrich ciliate protozoa are able to attach to substrates containing soluble carbohydrates. This occurs via chemotaxis. This way, they could utilise the monosaccharides glucose, fructose, galactose, some soluble oligomers and polysaccharides, composed by one or more of these simple sugars (25).

The results demonstrate that the inclusion of Optigen in the ration of experimental animals results in changed in the total rumen ciliates counts. The size of ciliate populations was reduced at all study intervals. Particularly marked changes were observed in the hours after the morning feeding, when total counts decreased statistically significantly ($p < 0.01$) from 155.21 $10^3/cm^3$ to 95.54 cm^3 by post feeding hour 2.5 and from 219.42 cm^3 to 110.58 cm^3 by post feeding hour 5.

Rumen ciliates are involved in rumen fermentation, degrading dietary proteins and carbohydrates by means by secreted extracellular enzymes. The intricate relationships between rumen bacterial and ciliate populations also have an effect on the metabolism of nutrients.

In their studies, (6) proved that rumen ciliates played an important role in the hydrolysis of vegetable hemicellulose fractions. The investigations of (34) confirmed the assumptions that holotrich and entodiniomorph ciliates possess hemicellulase activity.

Ciliates are also responsible for a significant share of rumen content proteolysis. The available proteinases and peptidases are produces by ciliates, bacteria and fungi (9).

The existing antagonism between both microbial species, especially with regard to carbohydrate and protein substrates in the diet is often used as an indirect marker for evaluation of the effect of a given ration type on the size of these microbial populations.

We hypothesise that the reduced total counts of rumen ciliates was probably resulting from the increased growth of proteolytic bacterial colonies. The data about total ciliate counts indicate that the inhibiting effect became more pronounced with time passed after feeding, i.e. with advancing degradation of protected protein included in Optigen.

As to the generic composition of rumen microflora, our studies confirmed the classic tendency for highest proportion of ciliates from Entodinium sp. Relatively low shares of Diplodinium sp., Epidinium sp., Ophryoscolex sp., Isotricha sp. ciliates were observed, while Dasytricha sp. was virtually absent from the examined observation fields with rumen content. A total lack of Ophryoscolex sp. was established, but the relative proportion of Epidinium sp. increased during the experimental period.

The changes in the percentage ratio of studied ciliate genera is attributed again to the impaired equilibrium and competing relationships both between ciliate genera, and between ciliate and bacterial populations under the influence of diet supplementation with Optigen.

CONCLUSION

The performed experiments and the results allowed making the following conclusions about the effect of the Optigen dietary supplement applied at a daily dose of 12 g per animal on rumen parameters:

- The supplementation of yearling rams' ration with Optigen increased and stabilised rumen pH values, which varied within a narrow range – from 6.61 to 6.78. The differences between control period and post feeding hours 1, 2.5 and 5 were statistically significant ($p < 0.001$, $p < 0.05$; $p < 0.01$).
- Supplemented to the ration, Optigen reduced the total volatile fatty acids and ammonia concentrations in the rumen content after feeding ($0.05 < p < 0.001$).
- The cellulolytic activity was 1.3 times higher (9.02% vs 6.98% during the control period).
- Optigen exhibited an inhibitory effect on ciliate fauna ($p < 0.01$)

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EFFECT OF OPTIGEN ON SOME PARAMETERS OF RUMEN FERMENTATION IN YEARLING RAMS

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ABSTRACT

A physiological experiment was conducted to evaluate the addition of Optigen® to the ration of yearling rams on rumen fermentation. Optigen is a source of specifically protected non-protein nitrogen with controlled (slow) release in fore stomachs, specially designed for supplementation of ruminants' diets. Six yearling rams were used - Blackhead Pleven×Suffolk crosses, with initial live body weight of 45 ± 2 kg. The animals were of uniform gender, body weight and origin. They were housed indoor, in individual boxes at the Experimental base of the Physiology Unit to the Faculty of Agriculture, Trakia University – Stara Zagora. The experiment consisted of two periods – control and experimental with a 10-day period between them for adaptation of experimental animals to the new ration. The following parameters were investigated: hydrogen ion concentration (pH), ammonia concentration, total volatile fatty acid (VFA) concentration, total counts and generic composition of rumen ciliates, and in vivo cellulolytic activity in the rumen. It was established that the supplementation of yearling rams' ration with Optigen increased and stabilised rumen pH values, which varied within a narrow range – from 6.61 to 6.78. The differences between control period and post feeding hours 1, 2.5 and 5 were statistically significant ($p < 0.001$, $p < 0.05$; $p < 0.01$). Optigen reduced the total volatile fatty acids and ammonia concentrations in the rumen content after feeding ($0.05 < p < 0.001$). Optigen exhibited an inhibitory effect on ciliate fauna ($p < 0.01$) as well as 1.3 times higher cellulolytic activity (9.02% vs 6.98% during the control period).

Key words: Optigen, rumen fermentation, sheep digestion