RESEARCH ARTICLE **(Open Access)**

Changes in Blood Biochemical Indices in Yearling Rams after Dietary Supplementation of Optigen

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Abstract

A physiological experiment was conducted to evaluate the effect of the technological product Optigen® supplemented to the feed of yearling rams at a dose of 12 g. The blood concentrations of glucose, creatinine, urea, total protein, albumin, globulins, and protein coefficient (albumin/globulin ratio) were assayed in samples collected from *v. jugularis.* A positive influence of Optigen was established, with maintaining higher blood glucose, creatinine and urea levels 2.5 h after feeding ($p \leq 0.05$). The increased total protein concentrations during the experimental period suggested for enhanced and stable rates of absorption from the digestive tract into the blood – effect of Optigen's active principle. Higher albumin/globulin ratio ($p<0.001$) was demonstrated, as a result of increased albumin levels especially at post feeding hours ($p<0.001$) and the reduced blood serum globulins ($p<0.05$). The supplementation of Optigen to the ration did not show any side or adverse effect on the health and welfare of experimental animals.

Keywords: blood indices (Optigen), feeding additives, sheep.

1. Introduction

The analysis of blood parameters is an important means for assessment of the nutritional, health and welfare status of farm animals. After detailed analysis, they could be a good criterion for the evaluation of effects of different feeding practices (22). The knowledge of all digestive processes occurring in the complex stomach makes possible their modification with regard to the complete conversion of feeds by ruminants and increased productivity. During the last years, a special attention is paid on the possibilities for guided rumen fermentation through biotechnological products and enzyme preparations on the basis of non-pathogenic bacteria and yeasts. The ideal supplement should be specific and with stable effect, not to be absorbed in the digestive tract, not toxic for the host at the recommended dose, without residues in tissues and to be biodegradable when excreted in the environment (1). Many substances used so far – ionophore antibiotics, methane inhibitors, growth promoters etc. are not compliant with these requirements. The production of special nutritional supplements containing protected proteins has started. One of these products is Optigen. According to the manufacturer Alltech, USA, the active ingredient of Optigen® is covered with biodegradable film permitting controlled slow release of nitrogen. The material is an excellent nitrogen concentrate, which behaves completely differently from urea and could improve rumen fermentation through nitrogen supplementation(7,23). In our country, the studies on the effect of Optigen® as a dietary supplement in small ruminants are still scarce. The mechanisms of its biological action is neither known. The main purpose of the present study was to throw light on these points. A more general aim was to optimise the costs related to protein ingredients in the ruminant diets.

2. Material and methods

The experiment was conducted with six yearling rams, Pleven Blackhead × Suffolk crosses at the Experimental Base of Animal Physiology Unit to the

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(Accepted for publication March 25, 2015)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Faculty of Agriculture, Trakia University - Stara Zagora. The gender, body weight (average body weight at the beginning of the experiment 45±2 kg) and origin of animals were uniform. The animals were housed indoor, in individual boxes with constant access to drinking water and salt licks. The welfare of the experimental animals was considered with the rules introduced by European (5) and National (6) Directives. The animals were raised considering all the hygienic parameters, in this direction.

The experiment comprised two periods: control and experimental. During the control period 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 0.93 g.

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

		Chemical composition					
Feed	kg	DM, kg	FUG	PDI, g	BPR, g	Ca, g	P, g
		1,61	1,54	90	0≥	6,8	3,5
Meadow hay	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
Sunflower meal	0,17	0,15	0,14	19,89	21,93	0.70	1,84
				Daily intake			
		1,54	1,54	140,89	0,93	7,54	6,13

During the experimental period, the ration was supplemented with Optigen at a daily dose of 12 g per animal, the sunflower meal was excluded and barley mesh amount – increased by 100 g (Table 2). 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 12g Optigen® during the experimental period, the rumen protein balance changed insignificantly remaining within the reference limits of 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 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.

Table 2. Chemical composition of the ration for yearling rams with body weight of 45 kg . Experimental period

		Chemical composition					
Feed	kg	DM, kg	FUG	PDI, g	BPR, g	Ca, g	P, g
		1,61	1,54	90	0≥	6,8	3,5
Meadow hay	1	0,87	0,6	64	-3	6,53	2,26
Barley mash	0,7	0,61	0,94	66,5	-21	0.36	2,37
Optigen	0,012				24,6		
				Daily intake			
		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

Blood samples for analysis were collected from *v. jugularis* twice during both periods – prior to feeding and 2.5 h after feeding, in two consecutive days. All procedures were performed as per the requirements of the Trakia University Animal Ethics Committee. A 10-day adaptation period to rations was allowed. The following blood parameters were assayed: blood glucose, creatinine, urea, total protein, albumin, globulins, protein coefficient (albumin to globulin ratio). Blood samples were analysed on an automated analyser SYNCHRON CX9 PRO at the licensed lab Bodilab Ltd, Sliven.

The results were statistically processed by statistical software (Statistica for Windows). Variables are presented as mean values ± standard deviation (SD). For comparison of different parameters the one way ANOVA test was used.

3. Results and discussion

During the control period, blood glucose decreased in postprandial hours (from 4.39 to 4.01 µmol/L 2.5 h after feeding; $p \leq 0.05$), while during the experimental period, it increased to attain 4.50 µmol/L after feeding (Fig.1). Although the difference was not statistically significant, it corresponded to the established effect of Optigen on rumen fermentation processes – reduction in total volatile fatty acids (unpublished data). It is known that in ruminants, more than 30% of liver glucose is synthesised from amino acids(2) , maximum 10% from glycerol(2) and from 36% to 76% - from propionic acid(10). Our results showed clearly that despite the addition of protected protein, blood glucose was close to the upper reference range for the species.

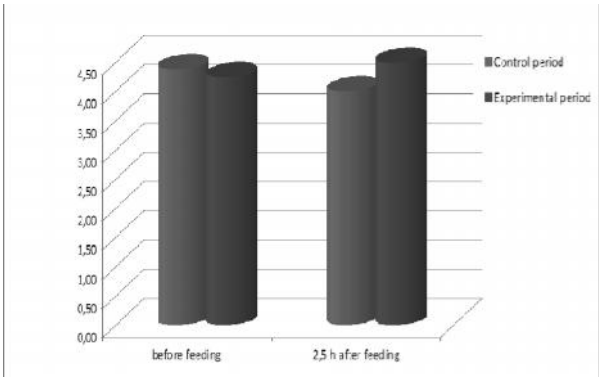

Figure 1. Blood glucose concentrations (mmol/L)

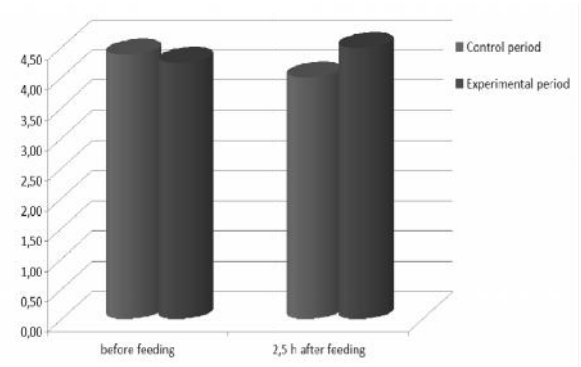

Figure 2. Blood creatinine concentrations (µmol/L)

Unfortunately, the studies in sheep are few and we could only make comparisons with reference values, which in sheep are between 2.8-4.4 µmol/L to conclude that our results were within the normal range. In the present study, blood creatinine (Fig. 2) ranged between 79 and 85 µmol/L, which is slightly above the lower limit of the reference range (76-184 µmol/L. The slight increase in blood creatinine after addition of Optigen both before and after feeding was statistically insignificant and within the physiological norms.

The one-way trend towards increased postprandial blood urea during the two study periods was a normal phenomenon and was accompanied by values remaining within the reference range. The effect of Optigen was clearly outlined by increased urea concentrations, with statistically significant difference by hour 2.5 after feeding ($p \leq 0.05$). The comparison of blood glucose and urea makes the overall picture complete. – The addition of a synthetic dietary nitrogen source induces higher blood urea concentrations (Table 3). The milder effect of Optigen supplementation is a natural sequel from nitrogen protection and thus, its slower release ensures gradual nitrogen release.

Plasma proteins are among the most important blood parameters. According to present results (Tab. 3) blood total protein values during both periods were very comparable, and within the reference range. The addition of Optigen resulted in weak and insignificant increase in total blood protein in experimental animals, more pronounced in fasting hours. Sivkova (13) established that the increase in dietary sugar beet proportion resulted in lower blood total protein both before and after feeding. Similar data about blood total protein in lambs and yearling sheep were reported by Grigorova et al. (8) and Todorova et al. (18). After addition of enzyme preparations Hostazym C100 and Hostazym X100 to the feed, a tendency towards increased blood total protein was demonstrated. Albumin is a primary source for protein synthesis in organs. Table 3 depicts data about blood

albumin levels in experimental animals. The values ranged within a narrow range (15-24 g/l) and exhibited opposite trends during the control and experimental periods. This could be attributed to the supplement Optigen, which increased blood albumin through the duration of the experiment, with statistically significant differences before feeding ($p \leq 0.001$). In a series of experiments, it was demonstrated that blood albumin in yearling sheep was influenced by dietary supplementation with enzyme preparations, and according to Caldiera et al. (4) albumin and urea are the best parameters of systemic protein metabolism in ruminants. Similar low blood serum albumin (15.1-15.8 g/l) values were reported in previous studies of ours, using Paulownia leaves as sole feed (21) and after testing the effect of Zarnela addition to rations.

The effect of Optigen supplementation to blood globulins was opposite (Table 3). During the control period, the values before and after feeding were 54.25 g/l and 50.83 g/l, and the decrease 2,5 h after feeding was not significant. After addition of Optigen, blood globulin was lower and stable (about 49 g/l), with statistically significant differences in pre-feeding hours ($p \leq 0.05$). The results were close to the upper reference range for sheep (35-55 g/l). They were equally comparable to those of other researchers and data from previous studies of ours (10,13,14,15,16).

The protein coefficient (albumin/globulin ratio) was again rather lower than the reference values (Table 3). Low blood serum albumin were the cause of that result. The lowest values were in pre-feeding hours during the control period (0.28), an the highest – after Optigen supplementation (0.50) with statistically significant differences ($p<0.001$). It was also found out that during the control period, the protein coefficient increased in postprandial hours, whereas after addition of Optigen the tendency was reversed, mostly by reason of maximum serum albumin value and the lowest globulin concentrations before feeding.

Table 3. Total protein, albumin, globulins and urea in blood serum

Period	Hour of study				
	before feeding			2,5 h after feeding	
	n	x	$\pm S_x$	x	$\pm S_x$
Urea (referent indices 2,6-6,6 mmol/l)					
Control period	12	4,23	0,35	4,57	0,28
Experimental period	12	4,44	0,20	5.52* ■■	0.25
Total blood protein (referent indices 60-80 g/l)					
Control period	12	69,42	1,81	71.25	1,41
Experimental period	12	72,92	0,78	71,50	0.82
Albumin (referent indices 25-39 g/l)					
Control period	12	15,17	0,56	20,42 ■■■	1,21
Experimental period	12	24.08***	0,83	21,75	1,41
Globulins (referent indices 35-55 g/l)					
Control period	12	54,25	1,78	50,83	1,70
Experimental period	12	48,83 *	0,82	49,75	1,37
Albumin/Globulins Ratio (referent indices 0.709-0.714)					
Control period	12	0,28	0,01	0,42 ■■	0,04
Experimental period	12	0,50***	0,02	0,45	0,04

*-Statistical significance between control and experimental period

■ - statistical significance between before and 2.5 hours after feeding *

■ - $p<0,05$; **, ■■ - $p<0,01$; ***, ■■■ - $p<0,001$

Similar results were obtained in previous studies in which the addition of Zarnela (dried distillers grains with solubles, by-product of wheat-based ethanol production in the ethanol producing enterprise) increased protein coefficients (A/G ratio) up to 0.310, *vs* control values of 0.291-0.294–and throughout the experiment, the protein coefficient attained only 60% of the lower reference limit of 0.48 (3).

4. Conclusions

It was concluded that the supplementation of protected nitrogene to the ration of yearling rams does not show any side or adverse effect on the health of experimental animals. Moreover, this cause an increase and maintenance of stable and higher blood glucose, creatinine and urea levels 2.5 h after feeding. At the same time, this increases protein coefficient values as a result of increased albumin levels and the reduced blood serum globulins, especially at post feeding hours.

5. References

1. Beauchemin, KA, Yang WZ, Rode LM (1999). **Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows**. J Dairy Sci, 82: 378-390.

2. Bergman, EN (1973). **Glucose metabolism in ruminants as related to hypoglycemia and ketosis**. Cornell Vet, 63:341-362.

3. Boyd JW (1984). **The interpretation of serum biochemistry test results in domestic animals. In: Veterinary Clinical Pathology, Veterinary** Practice Publishing Co, 13(2).

4. Caldiera RM, Belo CC, Santos CC, Vazques MI, Portugal AV (2007). **The effect of body condition score on blood metabolites and hormonal profiles inewes**. Small Rumin Res, 68:242-255.

5. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on: T**he protection of animals used for scientific purposes**, Official J EU, L 276/33

6. Directive № 20 of November 1, 2012. **The minimum requirements for the protection and welfare of experimental animals and the requirements for establishment, cultivation and/or delivery**, GG num. 87 of 9 November 2012.

7. Garcia-Gonzalez, R, Tricarico JM, Harrison GA, Meyer MD, McLeod KM, Harmon DL, Dawson KA (2007) Optigen® **II is a sustained release source of non-protein nitrogen in the rumen**. J. Anim. Sci. 85 (Suppl. 1):98, Optigen-025-RT.

8. Grigorova N, Varlyakov I, Slavov T, Enev E (2010) **Effect of Hostazym C 100 and Hostazym X100 on some hematological indexes of yearling rams**. J Animal Sci (Bg), XLVII, 3, 33-41.

9. McAliister T, Hristov A, Beauchemin K, Rode L, Cheng K (2001). **Enzymes in ruminant diets. In Bedford, M. R. and G. G. Partridge (eds). Enzymes in farm animal nutrition**. CABI Publishing, pp. 273-298

10. Radev V, Slavov T, Enev E, Varlyakov I (2011). **Effect of coconut oil on rumen and duodenal ammonia concentrations and some blood biochemistry parameters in yearling rams**. Agricult Sci Technol, 3:224-228.

11. Santos J, Pereira MN, Dias Junior GS, Bitencourt LL, Lopes NM, Siecola Junior S, Silva JRM (2009). **Response of lactating cows to partial replacement of soybean meal by Optigen® or urea.** Proceedings of Alltech's 25th Annual Symposium, May 17-20, 2009, Lexington, KY. Optigen-061-RT.

12. Seal CJ, Reynolds CK (1993). **Nutration implications of gastrointestinal and liver metabolism in ruminants**. Nutr Res Rev, 6, 185-208.

13. Sivkova K (2007). Digestion process by the ruminants in the dependence of diet composition and structure. DSc Dissertation, Trakia University, Agricultural Faculty, Stara Zagora. (Bg)

14. Sivkova K, Enev E, Radev V, Nedeva N, Slavov T (2007). **Effect of the diet structure on some rumen, duodenum and blood parameters of carbohydrate exchange in sheep**. Animal Sci (Bg), 6:61-68.

15. Slavov T, 2013. Effect of dietary lipids supplementation on digestion, metabolism and productivity of small ruminants. Ph.D Dissertation, Trakia University, Agricultural Faculty, Stara Zagora. (Bg)

16. Slavov T, Radev V, Sivkova K, Varlyakov I (2011). **Effect of dietary coconut oil supplementation on some blood biochemical indices in yearling rams**. Agricult Sci Technol, 3: 313-317.

17. Stewart, RL Jr, Tricarico JM, Harmon DL, Chalupa W, Mcleod KR, Harrison GA, Clark LM, Garcia-Gonzalez R, Dawson KA (2008). **Influence of Optigen® on nitrogen behavior in lactating dairy cows**. J. Dairy Sci. 91 (E-Suppl. 1):491, Optigen-059-RT.

18. Todorova P, Grigorova N, Radev V, Sivkova K (2009). **Effect of the multienzymatic preparation Hostazym X 100 on the weight development, forage utilization and some hematological characteristics in lambs**. Proceedings of IV International Conference Balnimalcon – 2009, 14-16 May, Stara Zagora, 228-232.

19. Tosev A (1975). On some processes in the rumen and blood composition of ruminants depending on ration composition and structure, DSc Dissertation, Stara Zagora.(Bg)

20. Varlyakov I, Radev V (1998). **Evaluation the effect of adding Yea Sacc 1026 at the diet of lambs by ethological, microbiological and hematological parameters**. Proceedings of Jubilee Scientific Session, November 1988, Plovdiv, vol. II, 361-364.

21. Varlyakov I, Radev V, Slavov T, Ganchev G (2013). **Blood parameters in yearling sheep feed Paulownia (Paulownia spp.) leaves**, Agricult Sci Technol 5:405-409.

22. Yurtman IY, Polatsu Ş, Başpinar E, Özdüven ML (1997). Farkli Ham Protein Düzeylerinin Yoğun Besideki Türkgeldi Erkek Kuzularında Bazı Kan Metabolitlerine Etkileri. Veteriner Fakültesi, Veteriner Bilimleri Dergisi, 13(1): 17-24 (Tu).

23. Wahrmund J, Hersom M (2007). **Evaluation of Optigen® II addition to by-product supplements for forage-fed beef cattle**. J. Anim. Sci. 85(Suppl. 1):410, Optigen-026-RT.