Original paper

EFFECT OF RAPESEED MEAL AS PROTEIN SUPPLEMENT IN FEEDLOT LAMB DIETS ON EXCRETION OF URINARY PURINE DERIVATES AND MICROBIAL PROTEIN SYNTHESIS

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Abstract

The objectives of our work were to establish the feeding effects of rapeseed meal (*RSM*) on urinary purine derivatives (*PD*) excretion as an indicator of ruminal synthesized and yielded microbial crude protein (*MCP*), and nitrogen (*N*) balance. Experimental animals (n=5, Tsigai breed wethers, 4-y aged, 65±2kg *BW*) averaging 1.1±0.1kg digestible organic matter intake (*DOMI*) randomly assigned to dietary treatments (n=2) in the two-stage 21–d periods– preliminary (14-d) and collection (7-d). Diets were consisted of 36% meadow hay, 34% cereal (corn, tritikle), 3% vitamin–mineral premix, 27% protein supplement. Diets were either control (with sunflower meal=*SFD*) or experimental (with rapeseed meal=*RSD*). They're iso-nitrogenous (crude protein, *CP*=16%), iso-caloric (net energy as feed units for gain, *FUG*=1.0/kg *DM*), equal in protein digestible in small intestines (*PDI*=86g/kg dry matter) and *Ca:P* ratio (2:1). The results have revealed that *RSD* gained (p=0.2) in higher *DOMI* (1.1 vs. 1.0kg) and tended to improve *N* balance (p=0.4) compared with *SFD*. *PD*-s value (24.0 vs. 22.3Mm), as allantoin (14.8 vs. 11.7Mm), xanthine+hypoxanthine (7.2 vs. 8.6Mm) and uric acid (1.9 vs. 2.0), respectively weren't affected (p=1.0, 0.9, 0.5 and 0.7, respectively) among the dietary treatments (*SFD* vs. *RSD*). Similarly, established ratios, as allantoin (2.1 vs. 1.9) and *PD* to creatinine (2.4 vs. 2.1, respectively), were not differ (p=0.3, respectively) between diets (*SFD* vs. *RSD*). Evaluated microbial *N* yield (20.7 vs. 19.3g, respectively) wasn't affected (p=0.9), and gained 19.3 vs. 17.1 microbial protein synthesis efficiency, respectively (p=0.8). Observed relationship between *DOMI* level and allantoin value (R^2=0.62), and between *PD* value and *MCP* level (R^2=0.99) suggested good parity among the treatments. In conclusion, the usage of high level *RSM* (27% of total ration), as a protein source in feedloot lamb diets tended to improve *DOMI* and dietary *N* utilization, but didn't affect *PD* excretion and microbial *N* yield.

Key words: *dietary protein source, ruminants, microbial N, purine derivatives, rapeseed meal*

Abbreviations: *Ca*-calcium; *CP*-crude protein; *DM*-dry matter; *DOMI*-digestible organic matter intake; *FUG*-feed units for gain; *GE*-gross energy; *MCP*-microbial crude protein; *N*-nitrogen; *P*-phosphorus; *PD*-purine derivatives; *PDI*-protein digestible in small intestines; *RSD*-rapeseed meal–based diet; *RSM*-rapeseed meal; *SFD*-sunflower meal–based diet; *TR*-total ration.

Introduction

In regards to sustainable ecosystems policy and eco–agro–socio–economic efficiency (Yossifov, 2014) of diet balancing, new feeding strategies with alternative feedstuffs and optimization of productive systems are required.

So, the ruminant nutrition must meet the N requirements of host organism and ruminal microorganisms in regards to improve N utilization for animal performance and to limit N excretion (Tamminga, 1992). The microbial protein synthesis (*MCP*) must cover protein requirements in ruminants, especially high-productive animals (Tas and Susenbeth, 2007). But, the conversion of dietary protein into microbial protein and the microbial protein yield is related to some factors, such as feeding level and passage rate and available energy and amino acids (*AA*) in synchronous rate (Firkins et al., 2007). For higher and strong microbial efficiency, substrate energy must be transformed at short chain fatty acids (*FA*) and adenosine triphosphates molecules (*ATP*). So, diet nutrients must ensure energy and N supply to rumen microbes.

The MPC could be predicted by the concentration of renal excreted purine derivates (*PD*) in regards to relationship to MCP (Chen et al., 1992; Shingfield, 2000). The duodenal purine bases (*PB*) are used as a microbial marker as an efficiently absorbed and the majority of their derivatives excreted via the kidney. So, the ratio of PDs in urine closely reflects, and therefore may be used to predict, microbial protein flow.

The objectives of current work were to 1/ establish the effects of feeding rapeseed meal–based diets (RSD) on urinary purine derivatives (PD) excretion as an indicator of ruminal synthesized and yielded MCP, 2/ estimate nitrogen (N) balance, and 3/ draw relationship model between established parameters and among the treatments. We hypothesized that the usage of high levels rapeseed meal (27 % in total ration), as a protein source in sheep diets, would affect DOMI, N balance, PD excretion and microbial N yield.

Material and methods

Experimental design. Experimental units were conducted at Experimental Base of Institute of Animal Science, Kostinbrod, BG. Animals averaging 1.1 ± 0.1 kg digestible organic matter intake (DOMI = (Coefficient of OM digestibility x OM content) x Feed intake) randomly assigned to dietary treatments (n= 2) in the two-stage 21–d periods – preliminary (14-d) and collection (7-d) (adopted from Yossifov and Kozelov (2014). Wethers were housed in individual metabolic cages with free access of fresh water and were fed twice a day (8.00 and 16.00 h).

Animals and treatments. Five Tsigai breed wethers (4-y aged, 65 ± 2 kg BW) were used in an experiment. Diets were consisted of 36 % meadow hay, 34 % cereal (corn and tritikle), 3 % vitamin–mineral premix and 27 % protein supplement (Table 1). Dietary treatments were either control (with sunflower meal – SFD) or experimental – with rapeseed meal (RSD). Offered diets were iso-nitrogenous (crude protein, CP= 16 %), iso-caloric (net energy as feed units for gain, FUG= 1.0/kg DM), equal in protein digestible in small intestines (PDI= 86 g/kg DM) and Ca:P ratio (2:1).

Sampling and procedures. Feed, faeces and urine samples were quantitatively collected per animal during the collection period. Feeds were dried at 65 ^{o}C, ground and then analyzed

(AOAC, 2002) for chemical composition (DM, CP, ether extract (EE), crude fiber (CF), Ash). Consumption (feed and water) was calculated for each animal as a difference between offered and refused (if orts were presented). Total digestible nutrients (TDN), energy and protein values were investigated and reported elsewhere (Yossifov and Kozelov, 2014). The faeces samples were analyzed for total N. Urine samples were analyzed for total N and purine derivatives (allantoin, xanthine + hypoxanthine, uric acid) as described in IAEA (1997). The amount of microbial purine derivatives was calculated as discribed by Chen and Gomes (1995).

Calculations and biostatistics. Analyses of variance and simple linear regression were performed. All observed data were presented as mean with SEM. The treatment means were compared by Student's T-test and were accepted as representing statistically significant differences at $p < 0.05$ and trends were discussed at $0.05 < P < 0.20$. The deduced relationships between DOMI level (kg/d) and allantoin excretion value (Mm/d) and between PDs excretion value (Mm/d) and MCP level (g N/d) were prepared by MS Office Statistic Package.

Results and discussion

The composition of the experimental diets were similar for SFD and RSD (Table 1). The difference was observed at diet digestibility (Yossifov and Kozelov, 2014), affecting TDN (11 %) and ME (14 %) concentration among the treatments.

The higher digestibility of OM significantly improved the values of the digestible OM (*DOM*) for RSD than SFD ($p<0.05$). So, thus reflected on the intake of DOM (*DOMI*) among the diets trending ($p= 0.18$) with the positive effect for RSD (Table 2). This considers the effective digestibility of ruminant diets in depends on the amount of soluble, potentially digestible and indigestible fractions, as well as the rate of passage and their ruminal digestion (Van Soest, 1982). Increased *DOM* might be related to increased OM outflow from the rumen with a high proportion of bacterial cells being associated with the particulate matter. So, it is apparent greater flow of particles from the rumen at an early stage of digestion with more attached microbes (Faichney, 1980; Van Soest, 1982; Merchen et al., 1986).

The investigation on value of metabolizable energy (ME) tended to higher levels for RSD than SFD (14 %). Such higher levels must to reflect on the amount of microbial protein synthesized in rumen as an energy source.

The effect of dietary protein source on nitrogen balance (*N*) is presented below (Table 2). The between-animal variation in N balancing trial was markedly higher in the present study, so the differences were not significant and shown only trends. As shown, N intake (N_i) is similar between diets (N_i= 38.2 g/d). The N balance in terms of N available (N_a, $p= 0.7$) and urinary N (N_u, $p= 0.6$) also did not be affected by the treatments. Although, N retention (N_r) and fecal N (N_f) tended to be increased (14 %) by rapeseed meal supplementation ($p= 0.4$). Such trendline was observed among calculated N balancing ratios in RSD fed animals ($p> 0.05$) than SFD fed animals (Table 2). The strongest trend (15 %) was estimated for the values of utilized N per 1 g N_i ($p= 0.19$). In regards to N utilization some authors noted that N excretion and N retention should reflect the differences in N metabolism, but the latter index was the most important of the protein nutrition status (Zinn, 1988). Also, this data confirmed the opinion that N_u decreased in order of energy supply (Balch, 1967).

Table 1. -*Experimental design (%) and diet composition (%)*[*1]

		Treatments	
		SFD	RSD
Ingredient			
Meadow hay		36.6	36.3
Cereal			
Corn		17.2	17.0
Tritikale		17.2	17.0
Protein supplement			
Sunflower meal		26.3	-
Rapeseed meal		-	27.2
Premix[2]		2.7	2.5
Nutrient			
Dry matter		1584.1	1606.28
Organic matter		95.3	94.8
Digestible OM		73.3[a]	78.9[a]
Total digestible nutrients		61.5	68.0
Energy	Gross	17.9	18.0
	Net, as FUG	1.0	1.0
	ME[3]	2.2	2.5
Crude protein		16.4	16.5
PDI		8.6	8.6

[*]Adopted from Yossifov and Kozelov (2014); [1]as DM basis, except DM (g) and energy (GE (kcal), ME (Mcal) and FUG (units); [2]Vitamin-mineral premix + limestone + dicalcium phosphate; [3]Metabolizable energy (ME, Mcal/kg DM)= (TDN×0.04409×0.82); [aa]$p<0.05$; RSD-rapeseed meal- based diet; SFD-sunflower meal-based diet; PDI-Protein digestible in small intestine; FUG-feed units for gain.

The mean values for daily excretion of the PDs are shown in the table above (Table 2). The between-animal variation in PD excretion also was markedly higher in the present study, so the differences were not significant and shown only trends. The allantoin and PD excretion values decreased with 21 and 7 % feeding RSD. The RSD increased with 19 and 7 % for xanthine + hypoxantine and uric acid values, respectively. Also, insignificant reduction in PD excretion was observed when ME intake was higher in other reports (Giesecke et al., 1984).

As also shown for N and PD, the between-animal variation in efficiency of microbial protein synthesis (*EMPS*) was higher in the present study, so the differences were not significant and shown only trends. The *EMPS*, presented as different indices, in SFD and RSD fed animals was not altered ($p> 0.8$) with the exception of the N_i/DOMI values ($p= 0.3$). These values were similar to those obtained in sheep by other authors (White et al., 2002; Yu et al., 2002).

Table 2. *Effect of RSM vs. SFM supplementation on productive parameters (per day)*

Item	Treatments		SEM	P-value	r^2
	SFD	RSD			
DOMI, g/d	1.05	1.13	0.04	0.18	0.54
N balance, g/d					
N intake (N_i)	38.16	38.29	1.73	0.98	0.92
Fecal N (N_f)	9.21	8.16	0.59	0.46	0.98
Available N (N_a)	28.94	30.13	1.32	0.72	0.89
Urinary N (N_u)	13.17	12.21	0.75	0.60	0.99
Retention N (N_r)	15.77	17.93	1.09	0.42	0.88
N_r / N_i	37.03	42.57	2.61	0.38	0.88
N_r / N_a	54.48	59.15	1.98	0.33	0.97
Utilized N per g N_i	0.41	0.47	0.02	0.19	0.97
N_r/N_i per 100g CP/кg DMI	5.93	6.81	0.42	0.38	0.88
Renal Excretion, Mm/d					
Allantoin	14.83	11.72	4.92	0.54	0.96
Xanthine + Hypoxanthine	7.24	8.60	2.66	0.63	0.90
Uric acid	1.89	2.02	0.74	0.86	0.53
PD/ $BW^{0.75}$	1.05	0.98	0.18	0.85	0.93
Efficiency					
MCP, g N	20.65	19.28	4.48	0.90	0.94
N_i/DOMI (g/kg)	36.36	33.67	1.09	0.31	0.93
MCP N/DOMI (g/kg)	19.28	17.11	3.92	0.83	0.87
MCP N/ME intake (g/Mcal)	0.0064	0.0055	0.001	0.78	0.89
MCP N/N_a	0.68	0.67	0.12	0.95	0.87
Ratios					
Allantoin:Creatinine	2.13	1.88	0.44	0.25	0.95
PD:Creatinine	2.44	2.14	0.51	0.33	0.93

SFD-sunflower meal-based diet; RSD-rapeseed meal-based diet. DOMI-digestible organic matter intake; CP-crude protein; PD-purine derivatives; BW-body weight; MCP-microbial protein; ME-metabolizable energy.

The deduced relationship models between established parameters and among the treatments are presented below (Fig. 1 and 2). The graph of relationship between DOMI level ($kg.d^{-1}$) and allantoin excretion value ($Mm.d^{-1}$) is shown in Fig. 1.

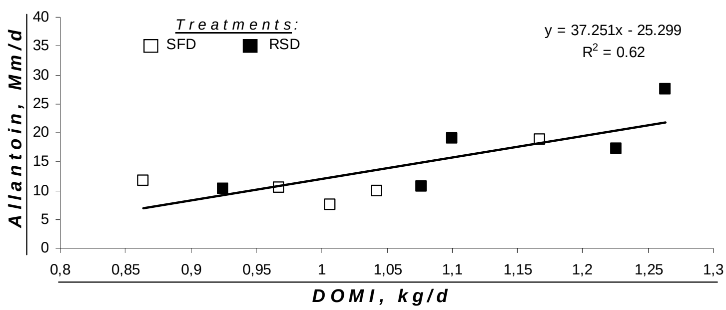

Figure 1. *The relationship between DOMI level (kg/d) and allantoin excretion value (Mm/d).*

Observed correlation suggested good parity among treatments (R^2= 0.62). Regression analysis indicated that the values of allantoin excreted (y) were closely related to estimated level of

DOMI (x). The slope of 0.1807 and small intercept of 0.1807 indicate that both parameters gave comparable results (Fig. 1). Such strong relationship confirmed the results of others (Perez et al., 1996).

The graph of relationship between PD excretion value (Mm/d) and MCP level (g/d) is presented in Fig. 2.

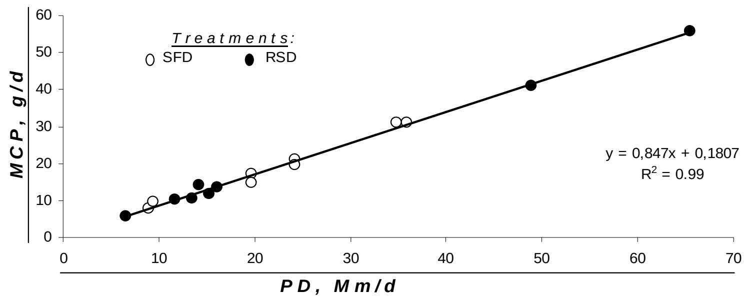

Figure 2. *The relationship between PD excretion value (Mm/d) and MCP level (g N/d).*

Observed correlation suggested good parity among treatments (R^2= 0.99). Regression analysis indicated that the values of microbial protein synthesized (y) were closely related to estimated level of PDs excreted (x). The slope of 0.1807 and intercept of 0.847 indicate that both parameters gave comparable results (Fig. 1).

Conclusion

Results from this study indicate that rapeseed meal (RSM) was superior to sunflower meal (SFM) in terms to having higher dietary digestible organic matter (DOM), total digestible nutrients (TDN) and metabolizable energy (ME), and to optimizing N utilization. Based on this research unit, it can be concluded that substitution of dietary protein source and diet supplementation with RSM vs. SFM improves diet composition as DOM, TDN and ME with 8, 11 and 14 %, respectively. The higher (8 %) digestible organic matter intake (DOMI) at rapeseed meal-based diet (RSD) compared to sunflower meal-based diet (SFD) optimizing N utilization by lower N excretion (9 %) and higher N retention (14 %) at equal N intake. The level of the purine derivatives (PD) excretion and efficiency of microbial protein synthesis has been not affected. Close relationships were found between DOMI level and allantoin excretion value, and between PD excretion value and MCP level. This study recommended RSM to be used as local feedstuff and alternative protein and energy supplement to improve animal performance at intensive feeding systems.

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