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THE INFLUENCE OF REPLACING SLOW WITH RAPID STARCH IN GROWING RAMS' DIETS ON THE LEVEL OF RUMEN MICROBIAL PROTEOSYNTHESIS

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Abstract: The objective of the study was to estimate the level of microbial proteosynthesis in sheep, following the replacement of a classical ingredient (corn) with a rapidly fermentable energy source (barley), when protein ingredient of the compound feed is highly degradable (rapeseed meal).The diets were tested on two groups of four Merinos rams each, weighing 50-55 kilos. Regular procedure for in vivo digestibility tests was used and urine was collected for determination of purine derivatives concentrations. The consumption of the two diets led to similar nutritional supplies: 1.26-1.29 MFU, 124-129 g IDPN, 112-118 g IDPE; the groups being distinguish only in terms of the dynamics of energy availability at the ruminal level. The amount of purine derivatives excreted in urine were 12.58 mmols/day in the corn group and 9.49 mmols/day in the barley group; consequently, the rumen microbial proteosynthesis was estimated at 43.11 g IDMP/day for the corn group and 31.3 g IDMP/day for the barley group (P=0,396). It is concluded that the effect of synchronizing the energy and protein dynamics in barley group was counteracted by the fact that energy and protein availability were limited to the first hours after administration of the compound feed, when the capacity of the ruminal microorganisms to grow was probably exceeded. In order to maximize their growth potential, it is necessary to extend the period of synchronized ruminal availability of energy and protein.

Key words: microbial protein, rumen, starch, barley, corn

Introduction

The capacity to accurately predict and to stimulate microbial protein supply is of prime importance in ruminant nutrition. It is unanimously acknowledged that

ruminants use very inefficient the nitrogen from common diets. The usual strategy aiming to ensure high animal performances is to feed diets exceeding protein requirements but, as the efficiency of its conversion to animal products is low, the excess nitrogen is excreted toward environment.

One of the reasons of this low efficiency is the lack of synchronicity of energy and protein supplies, which can occur at the level of the whole diet (difference between microbial protein allowed by nitrogen and that allowed by energy) or at the level of the dynamics of energy and protein availability in the rumen. The first one is more obvious, as it can be estimated within modern feeding systems, such as IDP *(Verite, 1988)***;** the second one requires taking into account more factors, such as rate of protein degradation, fermentability of energy, passage rate, etc. Because microorganisms digest the largest part of the ingested feeds, the interaction between carbohydrate and protein rumen metabolism is particularly strong. If there is a deficiency or inefficient utilization of the protein, the digestibility of carbohydrate can also decrease. If there is insufficient carbohydrate to match protein supply, nitrogen can be lost as ruminal ammonia *(Nocek and Russell, 1988).*

Therefore, an approach to ensure better use of dietary nitrogen would be to match the rapid or slower fermentable energy ingredients with their corresponding protein ingredients in order to provide a simultaneous release of the nutrients and simultaneous availability to rumen microorganisms. Thus, diets where protein source is more degradable may require rapidly fermentable energy in order to ensure synchronicity of protein and energy supply for microbial proteosynthesis.

The most important source of nonstructural carbohydrates is represented by grains, corn and barley being the predominant grains of Europe. The starch source may be of importance, because ruminal fermentation rates vary over a wide range, with barley being more rapidly fermented in the rumen than corn grain but less energy dense *(Yang et al., 1997).*

The objective of this study was to estimate the level of microbial synthesis in growing rams, when a classical ingredient of the diet (corn) is replaced by a source of rapidly fermentable energy (barley), while protein supply in the compound feed is ensured by a more degradable ingredient (rapeseed meal).

Materials and Methods

Eight Merinos rams, weighing 50 – 55 kg, housed in individual digestibility cages for sheep, allowing separate collection of urine and feces, were assigned randomly to two groups. During 10 days of adaptation and 6 days collection period, the animals were fed diets consisting of meadows hay (medium quality) and ground compound feed based either on corn (group 1) or on barley (group 2),

formulated according to *Burlacu, 1996.* The structure of the compound feed is shown in Table 1.

The diets were fed in one meal/day (8:30 a.m.), the compound feed preceding the meadows hay and the animals had free access to the drinking water. Consumption of hay and compound feeds was recorded daily and individually. Feeds samples were taken for proximal analysis, in order to estimate nutritive values of consumed diets.

Table 1. Structure of compound feed

	Group 1 (corn based diet)	Group 2 (barley based diet)
Corn, %	70.8	-
Barley, %	-	79.1
Wheat bran, %	3.5	2.4
Rapeseed meal, %	20.9	14.6
Calcium carbonate, %	3.5	2.6
Minerals & vitamins premix, %	0.9	0.9
Salt, %	0.4	0.4

Urine was collected according to *Chen and Gomez (1992).* Five-liter bins were used for individual and daily urine collection; sulfuric acid 10 N was used in order to maintain a low pH of urine samples. According to the authors of the method *(Chen and Gomez, 1992),* excess of sulfuric acid does not adversely affect the analysis of purine bases. After measuring the volume of urine collected over 24 hours, approximately 10% of the total volume was retained for each animal in plastic containers with screws that were placed in refrigerator, the rest being discarded. The daily retained volumes were pooled every two days; therefore for an experimental period of 6 days 3 samples for each animal were taken, leading to 12 observations per group. Of the two days pooled samples, 50 ml were placed in vials and frozen until analysis of purine derivatives.

Allantoin, uric acid, xanthine and hypoxanthine were assessed using a HPLC method. A high-performance liquid chromatograph JASCO - 980 was used and processing of chromatograms was performed with CHROMPASS software. Purine derivatives standards were prepared with ultrapure water and 1N sodium hydroxide solution was added until pH reached 7.8. Elution flow was 1 mL / min, detection was performed at 218 nm, column temperature was 25°C and filtration of samples was done on Teflon filters. Before determination of purine derivatives the urine samples were thawed. After checking whether the pH level is within the limits of the method, samples were stabilized with sodium hydroxide, passed through a 0.45 micrometer filter and injected into the HPLC column.

Results and Discussion

Whereas the meadows hay intake was about the same in both groups, the barley group ingested more compound feed than the corn group. However, the consumption of the two diets led to similar nutritional contributions: 1.26–1.29 mFU/day, 124–129 g IDPN/day, 112–118 g IDPE/day (Table 2) - the experimental groups being distinguished only in terms of degree of synchronization of energy and protein availability at ruminal level.

Table 2. Average consumption and nutritive supply of the experimental diets

	Group 1 (corn based diet)	Group 2 (barley based diet)
Consumption of diet ingredients (g/head/day)		
Compound feed	563.54 ± 79.15	701.94 ± 61.11
Meadows hay	942.13 ± 118.26	899.06 ± 76.67
Daily nutrient intake:		
DM, (g/day)	1294.6	1375.5
mFU/day	1.26	1.29
IDPN, (g/day)	128.5	123.7
IDPE, (g/day)	117.6	111.7
Ca, (g/day)	12.06	11.46
P, (g/day)	6.11	6.82

DM = dry mater; mFU=meat feed units; IDPN = intestinally digestible protein allowed by nitrogen supply; IDPE = intestinally digestible protein allowed by energy supply

Based on the urine volume and the concentrations of individual purine derivatives in urine samples, the total amount of excreted purine derivatives (PD) was calculated (Table 3).

Table 3. Daily output of urine and purine derivatives

Specification	Corn Group	Barley Group
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Urine volume, (ml)	761.875±58.9	556.450±41.8
Total allantoin, mmols/d	9.828±1.8	7.445±2.3
Total uric acid, mmols/d	1.778±0.2	1.195±0.4
Total xanthine, mmols/d	0.235±0.0	0.248±0.1
Total hipoxanthine, mmols/d	0.735±0.1	0.603±0.2
Total purine derivatives, mmols/d	**12.575±2.0**	**9.490±2.9**

Tendency for higher excretion of total purine derivatives was observed in animals that were fed the corn-based ration, comparing to those fed barley-based

ration: 12.57 versus 9.49 mmols/day (P=0.396). The PD concentrations obtained in the present study are lower than concentrations reported for sheep by *Richardson et al. (2003)* (ranging from 13.1 to 16.4 mmols/day) when a barley based ration is used.

Table 4 show the daily production of microbial protein, calculated from the amounts of purine derivatives excreted in urine using the systems of equations proposed by *Chen and Gomez (1992)*. On this basis, the quantities of synthesized microbial nitrogen were estimated at 10.78 g/d and 7.82 g/d (for corn and barley diets, respectively) leading to an average production of 43.11 g/d microbial protein in group 1 (corn based diet) and only 31.26 g/d in group 2 (barley based diet).

Table 4. Daily production of microbial protein

Specification		Corn Group	Barley Group
		$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$
BW, Kg		52.75±2.7	54.08±1.1
Purine derivatives in urine, mmols/d	Total	12.58±2.0	9.49±2.9
	Endogenous	0.12±0.1	0.46±0.2
	Exogenous	12.45±2.1	9.03±3.1
Purine derivatives in microbes, mmols/d		14.83±2.5	10.75±3.7
Microbial N, g/d		10.78±1.8	7.82±2.7
Microbial CP, g/d		67.36±11.3	48.85±16.8
IDMP, g/d		**43.11±7.2**	**31.26±10.8**

These results were contrary to the expectations, as in the barley group the dynamics of rumen availability of energy (supplied by barley) and protein (supplied by rapeseed meal) were better synchronized than in the corn group. In the later, the rapidly degradable source of protein (rapeseed meal) was associated with a slowly fermentable source of energy.

It is known that rumen-produced ammonia exceeding the capacity of ruminal microbes to incorporate it in the microbial biomass is lost *(Lobley et al., 1995).* It is possible that, in the barley group, the peaks of energy and nitrogen early availability exceeded the growth capacity of microbes in first post-prandial hours.

Another factor which may have interfered is the decrease of rumen pH for a certain period after the meal, which might had negative influence on microbial proteosynthesis.

The daily production of microbial N (10.78 g/d and 7.82 g/d) was greater than that recorded by *Witt et al. (1999),* who reported a mean value of 6.0 g/d, but was similar to those reported by *Henning et al.(1993), Sinclair et al. (1993, 1995)* and *Richardson et al. (2003)* in sheep fed at a similar level of intake.

Although there is general agreement that energy source is the primary influence on microbial protein production *(Stern et al., 1978; Henning et al., 1993;*

Shabi et al., 1998) there is still disagreement whether synchronizing dietary energy and protein supply to the rumen improves microbial protein growth.

In the current experiment, there was no significant effect of dietary synchrony on microbial yield. There's also a possibility that beyond a certain degree of synchrony, no additional response can be obtained. However, in general, increasing the energy level beyond an optimal level did not further increase microbial growth.

Conclusion

Replacement of corn (slower degradable starch) with barley (more rapid degradable starch) in compound feeds where protein ingredient was the rapeseed meal did not increase the microbial protein synthesis.

On the contrary, it seems that part of the dietary supply of both energy and protein in barley group was not used for microbial growth, because their peaks of ruminal availability, although synchronized, were limited in time leading to the exceeding of the growth capacity of the microbes.

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Uticaj zamene sporo- sa brzo- razgrađujućim skrobom u obroku za ovnove u porastu na nivo mikrobiološke proteosinteze u buragu

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Rezime

Cilj ovog ispitivanja je bio da se odredi nivo mikrobiološke proteosinteze kod ovaca, nakon zamene klasičnog sastojka (kukuruz) sa izvorom brzo fermentujuće energije (ječam), kada je protein komponenta u smeši visoko razgradiva (sačma uljane repice).

Obroci su ispitani na dve grupe od četiri ovna rase merino, težine 50-55 kilograma. Redovna procedura za *in vivo* testove svarljivosti je korišćena i urin sakupljan za određivanje koncentracija derivata purina. Konzumiranje dva obroka

su rezultirala u sličnim vrednostima: 1.26-1.29 MFU, 124-129 g IDPN, 112-118 g IDPE; grupe su se razlikovale samo u pogledu dinamike dostupnosti energije na nivou buraga.

Količina derivata purina izlučena u urinu bila je 12.58 mmols/dan u grupi koja je hranjena kukuruzom, i 9.49 mmols/dan u grupi koja je dobijala ječam; prema tome, mikrobiološka proteosinteza je procenjena na 43.11 g IDMP/dan za grupu hranjenu kukuruzom i 31.3 g IDMP/dan za grupu koja je dobijala ječam (P=0,396).

Zaključeno je da je uticaj sinhronizovanja dinamike energije i proteina u grupi koja je dobijala ječam za posledicu imala činjenicu da je dostupnost energije i proteina bila ograničena na prvih nekoliko sati posle administracije smeše, kada je kapacitet porasta mikroorganizama u buragu verovatno bio nadmašen. U cilju maksimiziranja kapaciteta za porast, veoma je važno producžiti period sinhronizovane dostupnosti energije i proteina u buragu.

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