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Proteosyntetic activity in the rumen of dairy cows

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Hight-quality microbial protein synthesized in the rumen of dairy cows represents the vast majority of the total quantity of amino acids entering the small intestine. (Sylvester, 2005). A study of factors affecting microbial profit and efficiency depends on the appropriate method of measuring proteosyntézy. Microbial protein has a high content of nucleic acids, consisting of nucleotides. Each nucleotide consists of pentosy, phosphate groups and nitrogenous heterocyclic purine and pyrimidine bases (Lindberg and Gonda, 1993; Tas and Susenbeth, 2007).

Measuring the flow of microbial protein in vivo requires surgically cannulated animals, which is costly and increases the labor and may have effect on dry matter intake and milk performance. Measurement of purine derivatives in urinary excretion is a non-invasive method. Their urinary excretion appears to be a credible method to determine the flow of microbial protein in the duodenum. The principle is based on the fact that amino acids and their derivatives in the duodenum are mainly of microbial origin, are largely spent and absorbed in the small intestine, purinové bases are catabolized on their derivatives and eliminated in the milk, sweat and urine. It is possible to determine the flow of nitrogen excretion of purine derivatives quantitative urine. Much of the purine derivative is eliminated in the form of allantoin (Tas and Susenbeth, 2007).

Absorbed in the body are metabolized to the purine derivatives - hypoxanthin, xanthina, uric acid and allantoin. Theoretically, the excretion purine derivatives should be related to the amount of microbial purines, and then the quantity of microbial protein absorbed from the small intestine (Kratky, 1997). The vast majority of total purine derivatives are excreted in the urine as allantoin (Tas and Susenbeth, 2007).

Methodology of experimental monitoring

Selected dairy cows were in the third lactation. Groups (breed Holstein - H and Czech speckled - C) and subsets (with low and with hight utilization) of cows were fed the same dose system TMR ad libitum. Feed for dairy cows have been made to feed the table. Number of dairy and feed places were 1 : 1. Feed table is equipped with an automatic corrector.

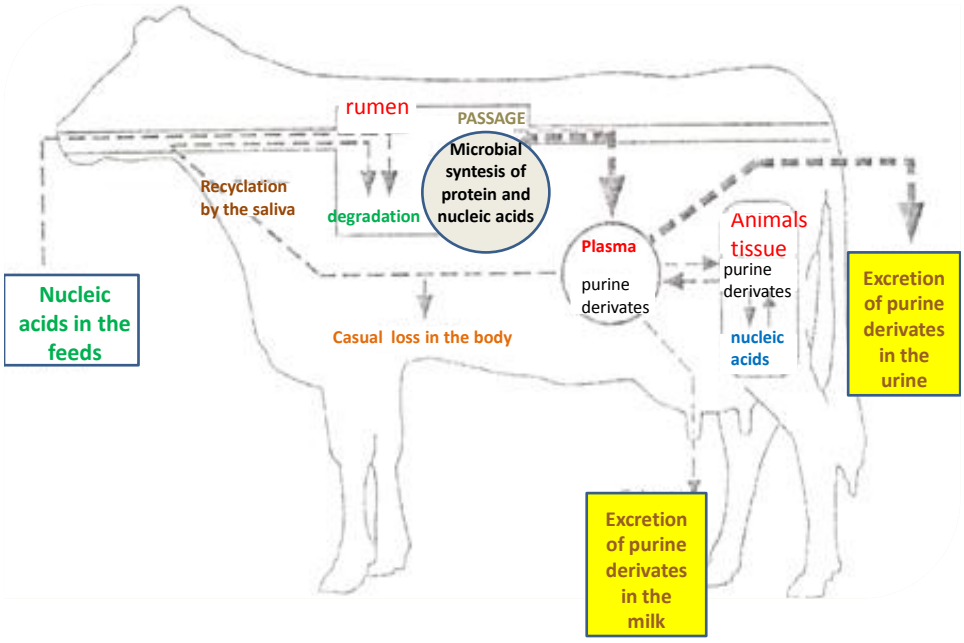
From each of the cows were repeatedly, always in one day collected 70 ml urine to PVC sample and added 15 ml H_2S0_4. The samples were stored at - 18 ° C.

Thawed sample was centrifugated 10 minutes at 10 000 speeds / min. Thus prepared samples were filtered and analyzed. Was used to determine the method proposed modification

George et al. (2005). Standards purines, and urea were prepared by dissolving the substance in a small volume of 1 M NaOH and supplemented with water in volumetric flask to a final concentration of 1 mg / ml. The concentration calibration curves were prepared using serial pils method. For the calibration curve, we used dilute solutions of 0.1 mg / ml, 0.5 mg / ml and 0.05 mg / ml of allantoin and uric acid. Allantoin and uric acid were determined using the RP-HPLC with UV detection, based on the method used previously Pineiro-Sotelo et al. (2002). Separation of bases achieved analytical HPLC system Oionex Summit (Oionex corp, U.S.), consisting of 680 P four chamber pump, diode array detector UVD340U, column thermostat coupled with a Waters 717 autosamplerem (Waters Inc., U.S.), using a Gemini C 18 column (2) (250 x 4.6 mm ID 5 mm particle size, Phenomenex, U.S.). The column was tempered at 35 ° C. Purins were analyzed under Isocratic 100% aqueous phase, using 50 mM KH_2P0_4 pH 4.0 as mobile phase flow of 0.8 ml / min. At the end of each test sample, the column rinsed with 20% acetonitrile for five minutes and is activated for a period of six minutes before further analysis. Under these conditions, sufficient separation of allantoin and uric acid was achieved after 16 minutes. Peaks were identified by comparing retention times and UV spectra of individual compounds, and finally using standard methods of blanc. Wavelength used to measure the UV maxima around each of the analytes (300 nm uric acid, allantoin 200 nm).

The calculation of microbial proteosyntesis we have valued under Verbic et al. (1990) adapted by Gonda et al. (1995). We compared two groups with two subgroups of dairy cows between them, to be absolutely level playing field, with a maximum exclusion of differences between the animals to watch and compare values.

Intake and excretion of purine derivates in dairy cows



Results and discussion.

Our results are not entirely in agreement with the authors claim Lindberg and Gonda (1993), that the excretion of purine derivatives endogene resources does not appear to be

affected by changes in energy intake and protein under normal conditions of feeding. Our dairy cows were fed ad libitně and the same ration and even though we can see that the same breed of dairy cows with a higher daily yield, and have higher total allantoin excretion in the urine of 15% compared with the group with lower daily performance, apply to breed H. By breed C to be similar. Authors Tas and Susenbeth, (2007) acknowledge that the purine derivative excretion may be affected by physiological state, which in our case is the level of lactation. This probably relates to the fact that allantoin excretion in urine is not only highly correlated with the flux of nitrogen into the duodenum, but also with the intake of dry matter (R^2 = 0.5), intake of organic matter (R^2 = 0.62), intake of digestible organic matter (R^2 = 0.62) as intake of nitrogen (R^2 = 0.91) (Stefanon, 1995). Firkins, (2006) notes that a large number of measurements (339 measurements), urinary allantoin excretion is dependent on the dairy cows in milk yield. This implies that income is related to dry matter yield and thus the intensity of proteosyntesis in the rumen (Hristov, 2004).

Our results confirm the assumption that the intensity of rumen proteosyntesis is influenced by the level of production. Production is limited by intake of nutrients and energy. Because dairy cows compared all groups and subgroups were fed the same dose of TMR system, the nutrient intakes were limited by dispensing power. Dairy cows with higher production received more nutrients to rumen micro-organisms to transform on his own body.

According to the method proposed Verbič, (1990) and modified Gonda, (1995), we calculated the quantity of biomass generated in the rumen per day and animal. In the group with lower daily productivity of the average daily production of the estimated 1247 or 1171 g of biomass. For groups with a higher yield was calculated 1708 or 1501 g of biomass per day per head. This is a 28 or 20% more than compared groups. This may be influenced by higher dry matter intake for dairy cows with a higher yield. The conversion of allantoin excretion to create biomass is only a rough estimate, because there is still neobjasněných number of factors in the metabolic pathways, and no accurate quantification of the flow of nitrogen could be an overestimation or underestimation proteosyntézy in the rumen (Tas and Susenbeth, 2007). In our case, but we did not want to define precisely the amount of biomass formed, but always compare the two, and two groups of dairy cows in different physiological conditions and we managed.

In the group with lower also with higher daily productivity of dairy, cattle breeds were represented, and C. H

While both breeds are used as a milking breed, it can be observed that the degree of genetic effects the formation of biomass in the rumen.

Content of feed ration and kontent of concentrate

Feeds	**kg**	**Concentrate**	%
Mays silage	13,00	Barley seed	16,00
Alfa-alfa hylage	18,00	Wheat seed CP 11,5 %	38,00

LKS silage	4,50	Soybean meal extr. CP48 %	19,00
Brewer's grains	6,00	Rapeseed meal extra.	20,00
Barley straw	0,60	$CaHCO_3$	2,00
Concentrate	7,00	Vitamins premix EX 14	4,00
Feed ration *(kg/kus)*	49,10	Meal of $Ca_3(CO_3)_2$	1,00
Dry mather of FR (%)	44,92	Dry mather (%)	89,37
Dry mather in the FR (g)	22057,00	Dry mather (g)	894,00
PDI-E (g)	2033,60	PDI-E (g)	122,60
NEL- (MJ)	133,14	NEL (MJ)	6,39
Calcium (g)	197,87	Calcium (g)	15,77
Phosphorus (g)	98,66	Phosphorus (g)	7,34

Excretion of purine derivatives and the calculated proteosyntesis on dairy cows under performance and breeding.

Dairy cows and their daily production	n	Breed	Daily milk in kg	Allantoin excluded	Uric acid excluded	Calc. daily production of biomass in g
Low d. production	10	H	16,8	1,47	0,23	1247
Hight d. production	10	H	38,6	1,68	0,37	1708
Low d. production	10	C	12,1	1,39	0,23	1171
Hight d. production	10	C	31,4	1,54	0,39	1501

Conclusion

From our observation and literature can be consulted to draw some general conclusions:

- Monitoring of microbial proteosyntézy in the rumen can be evaluated amount excluded allantoin in the urine of dairy cows.
- To compare rumen proteosyntézy can be used přepočtových factors established pursuant to the authors Chen, Verbič and Gonda.
- Dairy cows with higher yield, precluding a large number of allantoin in the urine, which can be concluded on a higher rumen proteosyntesis. It has been demonstrated in both controlled breeds.
- Proteosyntesis showed the differences between breeds rated Czech strakaté and Holstein, these were not significant.