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EFFECT OF SUNFLOWER EXPELLER SUPPLEMENTATION ON INTAKE AND DIGESTIBILITY OF PASTURE GRASS WITH LOW PROTEIN CONTENT

N. A. TODOROV[1 *] and H. S. ALI[2]
[1]*Thracian University, Faculty of Agriculture, BG-6000 Stara Zagora, Bulgaria
[2]Research Institute of Mountain Stockbreeding and Agriculture, BG-5600 Troyan, Bulgaria

Abstract

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The aim of this study is to estimate the effect of protein level in supplement to summer-autumn natural pasture on grass intake and digestibility in dairy cows. In 2 x 2 Latin square trials with two groups, three animals each are tested two supplements to dairy cows grazing typical Bulgarian natural pasture with 5.6% legumes. The first supplement was 5 kg cow^{-1} day^{-1} sunflower expeller (SUN) with 264 g kg^{-1} crude protein (CP), and the second one 6 kg cow-1 day^{-1} compound feed (COM) with 156 g kg^{-1} CP. Intake and digestibility of pasture grass was estimated by double markers - acid insoluble ash and chromium sesquioxide. SUN and COM digestibility was determined in classical *in vivo* experiment with wethers. Equations were developed for calculation of intake and digestibility of pasture grass when animal received supplemental feeds. The cows supplemented with SUN intake approximately 450 g more organic matter with pasture grass, compare to cows receiving COM ($P<0.05$). The digestibility of pasture grass was 0.575 - 0.598 and 0.545 - 0.575 respectively for supplementation with SUN and COM ($P<0.05$). Difference in intake and digestibility is due mainly on higher level of CP in DM of diet with SUN equal to 187 g kg^{-1} vs. 140 g kg^{-1} diet with COM. Modified markers method for estimation of intake and digestibility of pasture grass when animals received supplements is useful in research with grazing animals.

Introduction

Most of pasture grasses in the Bulgaria are with low protein content (Cheshmedjiev and Djarova, 1984). The reason is very low percentage of legumes in the sward (Todorova and Chourkova, 1998; Mishinev and Popova, 2000). The protein content decreased additionally during typical for the region dry summer and autumn, as well as with advance of vegetation and drying up of grasses (Martin Polo et al., 2003). The summer high temperature also influences negatively composition and digestibility of grasses (Fales, 1986; Buxton and Fales, 1994). Therefore, it is logical to increase protein in supplements during the summer and the autumn months grazing. H. S. Ali (2006, personal communication) came to the same conclusion analyzing production and economic results of 18 farms in region of Central Balkan Mountain. The effect of different supplementation of cows on pasture in this country is not investigated yet.

The aim of this study is to estimate the effect of protein level in supplement to summer-autumn natural

E-mail: ntodorov@tradel.net

pasture at the north foot of the Central Balkan Mountains on grass intake and digestibility in dairy cows.

Materials and Methods

Animals and Scheme of Experiment

The experiment was conducted with two groups, three cows each, using 2 x 2 Latin square schemes. The cows were assigned to different groups according to their age, live weight, date of calving, level of milk production and milk protein and fat content at the beginning of experiment. Experimental cows were crosses of the Brown Cattle breed, with live weight varying from 543 to 552 kg.

The trial was carried out in August and September, 2006. Before experiment cows were grazing the same pasture plot which was used during experiment, and were supplemented with 6 kg of the same compound feed used in experiment. Because cows were adapted to pasture grass and compound feed preliminary period before sampling faeces was only 15 days. We consider this period enough for adaptation to replacement of type of concentrate part of diet.

Two different supplements to the pasture contained mainly grasses and only 5.6% legumes were tested in experiment. The first one was 6 kg compound feed (COM) containing 156 g kg^{-1} crude protein (CP) and providing daily intake of 5.88 feed units for milk (FUM, one FUM is equal to 6 MJ net energy for lactation) and 940 g CP. The compound feed was comprised of 500 g kg^{-1} barley, 150 g kg^{-1} sunflower meal with 347 g kg^{-1} CP, 330 g kg^{-1} wheat bran, 20 g kg^{-1} mineral and vitamin supplements.

The second supplement consist of 5 kg sunflower expeller (SUN) containing 264.5 g kg^{-1} CP, 4.75 FUM and 1323 g CP. Cows receiving SUN were additionally supplemented with 30 g daily vitamin and mineral premix providing 60 000 IU vitamin À, 12 500 IU vitamin D, 400 mg irons, 400 mg magnesium, 500 mg manganese, 60 mg copper, 500 mg zinc, 4 mg cobalt, 4.5 mg iodine and 1.5 mg selenium. Quantity of vitamins and trace minerals in the premix is approximately equal to intake of vitamins and trace elements by the COM. The premix was given to the cows in the morning and evening individually in addition to the sunflower expeller.

Feed units for milk (FUM) and protein value (Protein, digestible in intestine and Balance of pr0tein in rumen) in both supplements were calculated on the basis of digestibility of feeds by weathers, according to Bulgarian systems (Todorov, 1997).

The cows received supplement in the barn whereabouts they were held tied and received individually only supplemental concentrate feeds and premix. From 8.30 a.m. till 6.00 p.m. they grazed on plot of 18 ha natural hilly pastures situated near to the farm (maximum distance 4.5 km). Experimental cows were on pasture together with another 14 cows.

Chemical composition of pasture grasses and two supplements according to the Weende-method (AOAC, 1996) are presented in Table 1.

Grass Intake and Digestibility

Intake and digestibility of pasture grass was determined with two markers. Acid insoluble ash (AIA) was used as an internal plant marker and chromium sesquioxide (Cr_2O_3) - as an external. External marker was given 10 days during each experimental period, following suggestion of Pigge et al. (1981) and Galyean et al. (1987), starting on day 8 of preliminary period. Cr_2O_3 is dosed in quantity of 3 g twice a day (1.5 g in the morning and 1.5 g in the evening). The quantity of chromium received daily was 2.052 g per cow. A single dose of Cr_2O_3 was included in a small piece of bread and set up in bolus. The boluses were given directly deeply in the mouth of cows.

Data for Cr in faeces and AIA in pasture grass and faeces was calculated in g.kg^{-1} organic matter to fit utilized equations.

Samples of faeces were taken by "grab sampling" directly from cows' rectum twice a day in the morning and in the evening, during the last three days (18th, 19th and 20th day) of each experimental period.

Samples of grass grazed by experimental cows were taken by hand. By careful and patient observation of a cow and estimation of the spot of grazing, the cow was driven off and grass cut by clippers from the spot of grazing. Samples were taken from all three cows three times during each period or a total of nine samples which were dried and mixed for analysis. Our ambition was to cut exactly the grass grazed by cows.

Digestibility of the grass was calculated on the basis of concentration of internal marker (acid insoluble ash) according to the equation:

$$\text{Digestibility} = 1 - \frac{\text{Marker concentration in herbage}}{\text{Marker concentration in faeces}}$$

Faecal output was calculated by the dilution of an orally-dosed external marker (Cr_2O_3) according to the equation:

$$\text{Faecal output} = \frac{\text{Marker dose rate}}{\text{Faecal marker concentration}}$$

Classical methods of calculations (Galyean et al., 1987; Mayes et al., 1995) were adapted for cases when, in addition to grazing, animals were receiving supplements with known chemical composition and digestibility.

Digestibility of COM and SUN was determined in a special experiment with wethers by the classical method of difference. In first experiment diet consisted of 467 g meadow hay plus 200 g kg^{-1} COM (or SUN) and the second experimental diet consisted of 467 g meadow hay plus 600 g kg^{-1} COM (or SUN). A calculation of digestibility was according description of Krusteva et al. (1983). Meadow hay origin from the region where cows was kept. Thus any interaction in digestibility is eliminated.

Following equations were applied for calculation of different parameters:

OMf (kg day^{-1}) = Ed/Ef (1)

OMfs (kg day^{-1}) = OMs (1 - dOMs) (2)

OMfg (kg day^{-1}) = OMf - OMfs (3)

Pfg (g day^{-1}) = OMf. cPf - OMs.cPs (4)

cPfg (g kg^{-1} OM) = Pfg/OMfg (5)

IOMg (kg day^{-1}) = OMfg.(cPfg/cPg) (6)

dOMg (fraction) = 1 - cPg/cPfg (7)

where:

OMf - organic matter in faeces, kg day^{-1}

Ed- external marker daily dose given to the cows, g day^{-1}

Ef - external marker concentration in faeces organic matter, g kg^{-1} OM

OMfs - organic matter in faeces, coming from supplement, kg day^{-1}

OMs - organic matter in supplement, kg day^{-1}

dOMs - digestibility of organic matter in supplement, fraction

OMfg - organic matter in feaces coming from grass, kg day^{-1}

Pfg - plant marker in faeces coming from grass, g day^{-1}

cPf - concentration of plant marker in faeces, g kg^{-1} OM

cPg - concentration of plant marker in grass, g kg^{-1} OM

cPs - concentration of plant marker in supplement, g kg^{-1} OM

cPfg - concentration of plant marker in feaces coming from grass, g kg^{-1} OM

IOMg - intake of OM from grass, kg day^{-1}

dOMg - digestibility of OM in grass, fraction

Energy Value of Grass

Gross energy (GE), digestibility of energy (dE), and digestible energy (DE) in pasture grass were calculated according to equation of Demarquilly et al. (1989, pp.198 - 199):

GE (MJ kg-1 OM) = 18.958 - 0.297 + 0.00726 CP (g kg^{-1} OM) (8)

Digestibility of energy (dE) as fraction was calculated from digestibility of OM (fraction) estimated in the experiment and equation:

dE (fraction) = 0.0957 dOM(fraction) - 0.007 (9)

Metabolizable energy (ME) in pasture grass was estimated according to equation of Vermorel (1989) for relation of metabolizable energy (ME) and digestible energy (DE) in the pasture grass:

ME/DE = 0.8417 - (9.9 x 10-5 CF) - (1.96 x 10-4 ÑP) + 0.221 x L (10)

where:

CF is crude fiber, g kg^{-1} OM in grass

CP is crude protein, g kg^{-1} OM in grass

L is level of feeding (maintenance is equal to one). In the experiment L was equal to 2.1.

Feed units for milk (FUM) in pasture grass were estimated according to Bulgarian energy system (Todorov, 1997).

Protein Value of Feeds

Protein value was expressed as protein digestible in small intestines (PDI) and balance of degradable protein in rumen (BPR) according to Bulgarian protein system (Todorov, 1997).

Digradability of protein in rumen, digestibility of protein in small intestine, and digestibility of ether extract (necessary for calculation of fermentable organic matter) was taken from Todorov et al. (2007, pp. 129 - 140). For the first experimental period were taken data of pasture grass in August, and for the second experimental period - in September.

Analytical and Statistical Methods

Proximate analysis of supplements, pasture grass

Table 1
Composition of feedstuffs and faeces*

Items	DM g.kg^{-1}	In dry matter, g.kg^{-1}									
		OM	CP	EE	CF	NFE	Ash	Ca	P	Cr	AIA
Feeds											
Pasture grass, I period	331	918.7	92.4	22.6	314.0	489.8	81.3	7.9	2.1		33.21
Pasture grass, II period	355 355	918.7	90.2	22.0	316.5	490.0	81.3	8.1	2.0		33.76
Sunflower expeller	898	930.3	294.6	151.5	317.7	166.5	69.7	3.7	8.1		12.33
Compound feed	881	953.6	177.9	41.6	89.9	644.2	46.4	7.2	7.1		11.09
Faeces											
Pasture + sunflower expeller											
I period, cow 3	128	868.8	106.9	30.3	333.7	397.8	131.2	15.6	5.3	0.447	61.13
I period, cow 13	132	869.2	102.2	30.6	334.8	401.6	130.8	16.1	4.8	0.444	61.32
I period, cow 23	133	867.7	102.1	30.9	328.7	406.0	132.3	15.0	5.2	0.444	61.28
Average I period	131	868.6	103.7	30.6	332.4	401.8	131.4	15.6	5.1	0.445	61.24
II period, cow 4	135	883.6	108.4	29.9	312.3	433.0	116.4	16.8	5.4	0.437	61.11
II period, cow 6	127	887.3	102.2	30.8	323.8	430.5	112.7	16.1	5.0	0.438	60.87
II period, cow 7	132	885.4	103.0	29.5	338.2	414.7	114.6	17.0	4.5	0.442	61.05
Average II period	131	885.4	104.5	30.1	324.8	426.1	114.6	16.6	5.0	0.439	61.01
Pasture + compound feed											
I period, cow 4	136	890.4	113.2	32.0	296.3	449.0	109.6	14.9	5.3	0.476	62.85
I period, cow 6	130	887.6	105.6	31.1	293.3	457.6	112.4	15.5	4.7	0.479	62.62
I period, cow 7	129	884.9	103.9	31.6	286.0	463.4	115.1	15.8	3.0	0.480	62.91
Average I period	132	887.6	107.6	31.6	291.9	456.6	112.4	15.4	4.3	0.478	62.79
II period, cow 3	130	876.4	108.4	28.5	287.9	451.6	123.6	17.2	5.2	0.471	62.58
II period, cow 13	135	876.1	111.9	28.9	289.9	445.4	123.9	16.7	5.9	0.469	62.40
II period, cow 23	136	872.4	116.0	28.6	297.5	430.3	127.6	18.6	5.4	0.463	62.00
Average II period	134	875.0	112.1	28.7	291.8	442.4	125.0	17.5	5.5	0.468	62.33

* Abbreviations in head of the table: DM= dry matter, OM= Organic matter, CP= Crude protein; EE=Ether extract; CF=Crude fiber; NFE=Nitrogen free extractives, Ca=Calcium, P=Phosphorus, Cr=Chromium, AIA= Aid insoluble ash

and faeces were carried out according to Weende method (AOAC, 1996), calcium - according to Shulz and phosphorus according to Gerike and Kurmis, as described by Krusteva et al. (1983).

Acid insoluble ash (AIA) was determined by the method of Van Kuelen and Young (1977), according to procedure À of BDS-ISO (2002). Treatment with hydrochloric acid by boiling for 15 minutes was made after ashing of samples. Thus, the unpleasant smell of faeces during evaporation of hydrochloric acid was reduced.

Chromium content in faeces was determined in residue after ashing of samples by atom absorption spectrophotometer (Perkin Elmer 1050, USA).

The significance between the average values of different indices was determined with t-test, using the software Statistica (2001).

Results

Chemical composition of feedstuffs and faeces is shown in Table 1. During both experimental periods, cows received 2.052 g chromium daily.

Digestibility and energy and protein value of two supplements is given in Table 2.

Average for three cows in group values for intake and digestibility of pasture grass, and data for their calculations are given in Table 3.

Cows' intake of pasture grass was bigger when supplemented with SUN in both experimental periods, compared to the supplement with COM. The differences of 0.455 and 0.413 kg intake of organic matter from the grass for the first and the second period, respectively, were significant at $P<0.05$. Cow intakes 7 - 8 % more pasture grass when supplemented with sunflower expeller, than with compound feed.

In spite of higher intake of pasture grass, grass digestibility was better in cows receiving SUN as a supplement, than in cows supplemented with COM. The difference in digestibility was 4.9 percent units during the first experimental period and 3.0 percent units in the second ($P<0.05$). Higher digestibility lead to higher energetic value of grass supplemented with SUN (0.826 to 0.866 FUM kg^{-1} organic matter), compare to grass supplemented with COM (0.775 to 0.782 FUM kg^{-1} OM) (Table 4).

The difference in grass intake and digestibility could be connected with protein level of diets. When compound feed with 156 g kg^{-1} CP was supplemented, CP in OM of diet was 139 - 140 $g.kg^{-1}$ and CP in DM of diet was 128 - 129 $g.kg^{-1}$ (Table 4), which was at the low limit of minimal requirements.

The balance of degradable protein in the rumen (BPR) for studied pasture grass was negative (Table 4). For the total diet BPR is also negative when pasture was supplemented with COM. This meant that there was a deficit of degradable protein for the normal development of microorganisms in the rumen, which cause lower digestibility of grass, compare to grass supplemented by SUN.

Discussion

Data of this experiment showed that high protein supplement such as sunflower expeller (SUN) to pasture grass with low protein content stimulated grazing of dairy cows and they had a bigger intake of grass

Table 2
Average digestibility and feeding value of supplements from three wethers

Items	Digestibility, as fractions						FUM/ kg feed	PDI	BPR
	DM*	OM	CP	EE	CF	NFE		$g.kg^{-1}$ feed	
Sunflower expeller	0.613	0.646	0.801	0.823	0.559	0.401	0.95	96.1	123
Compound feed	0.740	0.766	0.732	0.520	0.580	0.771	0.98	94.0	13.2

* Abbreviations DM, OM, CP, EE, CF, and NFE are as in table 1, FUM is feed units milk, PDI is protein, digestible in intestine, and BPR is balance of protein in the rumen

Table 3
Average data from analyses and calculations of intake and digestibility of pasture grass

Items (Methods of calculations)	I period		II period	
	SUN#	COM#	SUN	COM
OM# in supplements, kg day^{-1} (From table 1 and daily quantity)	4.177	5.040	4.177	5.040
Digestibility of OM in supplements, fractions (According to experiment with wethers)	0.646	0.766*	0.646	0.766*
Content of external marker in OM of faeces (Ef), g kg^{-1} OM	0.512	0.539	0.496	0.535
OM in faeces (OMf), kg day^{-1} (by equation 1)	4.007	3.807	4.137	3.835
OM in faeces from supplements (OMfs), kg day^{-1} (by equation 2)	1.479	1.179*	1.479	1.179*
OM in faeces from pasture grass (OMfg), kg day^{-1} (by equation 3)	2.528	2.628	2.658	2.656
Concentration of plant marker in grass (cPg), g kg^{-1} OM (from table 1)	36.15	36.15	36.75	36.75
Concentration of plant marker in supplement (cPs), g kg^{-1} OM (from table 1)	13.25	11.63	13.25	11.63
Concentration of plant marker in faeces (cPf), g kg^{-1} OM (from table 1)	70.50	70.74	68.91	71.23
Plant marker in faeces coming from grass (Pfg), g day^{-1} (by equation 4)	227.15	210.69	229.74	214.52
Plant marker in pasture grass g day^{-1}	231.4	213.9*	234.2	217.6*
Concentration of plant marker in faeces coming from pasture grass (cPfg), g kg^{-1} OM (by equation 5)	89.85	80.17*	86.43	80.78*
Intake of OM from pasture grass (IOMg), kg day^{-1} (by equation 6)	6.283	5.828	6.251	5.838
Intake of DM# from pasture grass, kg day^{-1} (by data in table 1)	6.839	6.344	6.804	6.355
Digestibility of OM in pasture grass (dOMg), fraction (by equation 7)	0.598	0.549*	0.575	0.545

SUN=Sunflower expeller, COM=Compound feed, OM=Organic matter, DM=dry matter

* -Differences between two type of supplementation are significant ($P < 0.05$)

even from pasture with relatively low residual mass, as on an experimental farm. The higher consumption of low quality late summer and autumn grass was connected with improvement of grass digestibility, as a result of better conditions for rumen fermentation and relief of space for new portion of forage. The favorable effect of increasing level of CP on digestibility of diet in stall kept ruminants is well known (Tyrrell and Moe, 1980; Zenton and Henrichs, 2007).

Several other authors established higher digestibility and intake of low quality pasture grass as a result of increasing level of protein in supplements (Kartchner, 1981; Lusby and Wagner, 1987; Strauch et al., 2001; Bodine and Purvis II, 2003).

Table 4
Energy and protein values of pasture grass

Items (Methods of calculations)	I period		II period	
	SUN#	COM	SUN	COM
Energy value				
Gross energy of pasture grass (GE), MJ kg^{-1} OM (by equation 8)	19.39	19.39	19.37	19.37
Digestible energy of pasture grass (DE), MJ kg^{-1} OM (by equation 9)	11.08	10.17	10.65	10.09
Metabolizable energy of pasture grass (ME), MJ kg^{-1} OM (by equation 10)	9.25	8.49	8.89	8.42
Metabolizability of energy of pasture grass (ME/GE), q	0.477	0.438*	0.459	0.436
Feed units for milk (FUM) in 1 kg OM of pasture grass	0.866	0.782*	0.826	0.775
Feed units for milk (FUM) intake with pasture grass	5.44	4.56*	5.16	4.52*
Protein value				
Crude protein in OM of pasture grass, g.kg^{-1} OM	100.6	100.6	98.2	98.2
Degradability of protein in grass, fractions	0.67	0.67	0.66	0.66
Digestibility of grass protein in small intestine, fractions	0.72	0.72	0.72	0.72
Fermentable OM in grass, g.kg^{-1} OM	554.0	505.0	531.5	501.5
PDI in grass, g.kg^{-1} OM	78.1	73.5	76.1	73.3
BPR in grass, g.kg^{-1} OM	-23.0	-15.9	-22.1	-17.7
Daily intake of PDI with grass, g	490.7	428.4	475.7	427.9
Daily intake of BPR with grass, g	-144.5	-92.7	-138.1	-103.3
Daily intake of CP with grass, g	632	586	614	573
Daily intake of CP with supplements, g	1323	940	1323	940
Daily intake of CP with whole diets, g	1955	1526	1937	1513
OM in whole diets, g	10.460	10.868	10.428	10.878
Crude protein in OM of whole diet, g.kg^{-1}	187	140*	186	139*
Crude protein in DM of whole diet, g.kg^{-1}	172	129*	171	128*

Abbreviations are as in table 2. SUN=Sunflower expeller, COM=Compound feed, OM=Organic matter, DM= dry mater

* -Differences between two type of supplementation are significant ($P < 0.05$)

Naidenova et al. (2005) found that 92 to 99% of changes in digestibility of grasses could be explained by changes in crude protein content. Each percent increase of crude protein in dry matter lead to increase of digestibility of dry matter of grasses by 1.58 to 1.99 %-units.

Bodine et al. (2000) found higher digestibility and intake of pasture grass when supplemented by soybean meal, then by maize.

Autumn grass needs higher protein level to achieve the same level of digestibility, compared to spring and summer grasses (Minson, 1990). This is supported by the effect of protein supplementation of autumn and winter pasture (Llewellyn et al., 2003a; Llewellyn et al., 2003b; Loy et al., 2003; Appeddu and Brown, 2003).

Results from this experiment shows that modified two markers method for estimation of intake and digestibility of pasture grass in cases when the animals received supplements is reliable and helpful in those common situations. Of course, the involvement of more steps of calculations with modified method is decreasing accuracy of results, compared to classical direct method of using markers in grazing animals without supplementation.

In vitro estimation of digestibility of supplements is easier. However, in vivo is possible to decrease or even to avoid associative digestibility by choosing appropriate forage in ration similar to pasture grass. In our case, hay from same area was used as a basic diet in differential digestibility experiment to determine digestibility of two supplements.

Conclusions

It was established that supplementation with sunflower expeller containing 264.5 g kg^{-1} crude protein increased intake and digestibility of autumn pasture grass, compared to supplementation with compound feed with 156 g kg^{-1} crude protein. The reason for differences is increased crude protein in diet from 129 to 167 g kg^{-1} in dry matter.

Equations were developed for calculation by two markers intake and digestibility of pasture grass, independently of supplementation with concentrate. They allowed the study to be carried out in real practical conditions.

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