

# The effect of undegradable crude protein supplementation on milk production and composition and reproduction of early-lactating cows

**I. Bruckental<sup>1\*</sup>, H. Tagari<sup>2</sup>, A. Arieli<sup>2</sup>, S. Zamwell<sup>2</sup>, Y. Aharoni<sup>1</sup> and A. Genizi<sup>3</sup>**

<sup>1</sup> *Institute of Animal Science and* <sup>3</sup> *Department of Statistics and Experimental Design, Agricultural Research Organization, The Volcani Center, Bet Dagan 50-250, Israel*

<sup>2</sup> *Department of Animal Science, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76-100, Israel*

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## ABSTRACT

One hundred and fifty six Israeli-Holstein cows were randomly allotted to two groups in a continuous design; of these, 59 cows entered the trial within 14 days postpartum. The trial lasted 75 days. Diet 1 (control group) contained 16.5% crude protein (CP), of which 11.0% was rumen-degradable (RDCP). Diet 2 (high-protein; HP) contained 17.8% CP; all the additional protein was planned to be rumen-undegradable. Cows were group-fed. Feed intake and milk yield were recorded daily and milk composition was analyzed every 2 weeks. Dry matter intake, milk, milk fat and protein yields (kg/d) of the control and HP treatments were 21.2 and 20.7 ( $P \leq 0.05$ ), 34.3 and 35.5 ( $P \leq 0.05$ ), 0.866 and 0.935 ( $P \leq 0.05$ ), and 0.963 and 1.002 ( $P \leq 0.05$ ), respectively. Average milk, milk fat and protein yields in the control and HP cows that entered the trial within 0-21 d after calving, were 37.0 and 39.6 ( $P \leq 0.05$ ), 0.992 and 1.043, and 0.985 and 1.056 ( $P \leq 0.05$ ) kg/d, respectively. Supplementation of additional undegradable CP at the beginning of lactation, tended to improve reproductive performance, which was attributed to the earlier recovery of body condition score of those cows.

**KEY WORDS:** undegradable crude protein, lactating cows, reproduction

\* Corresponding address

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## INTRODUCTION

Milk protein yield can be affected by the amount of CP flow into the small intestine (Clark, 1975; Girdler et al., 1987; Hof et al., 1994). In order to elevate available protein supply, undegradable protein must be added to the ration above the amount of microbial protein synthesised in the rumen. Ration carbohydrates and protein degradabilities should be synchronised in order to achieve maximal utilization of dietary CP for microbial protein synthesis (ARC, 1984; Arieli et al., 1989, 1993; Aharoni et al., 1993). In earlier studies carried out in our laboratory, the effective degradability of organic matter (EDOM) was used as an estimate of energy availability in the rumen (Arieli et al., 1993) and found to be positively correlated with the amount of CP leaving the rumen and with milk yield of the cows.

It has been proven in many experiments that during the first period after calving, cows respond to additional CP by increasing milk yield (Roffler et al., 1978; Bruckental et al., 1986, 1989). According to the NRC (1989), it is recommended to feed cows during the first 3 weeks after calving a ration containing 19% CP. However, if dietary CP composition is planned in terms of undegradable (UDIP) and degradable (DIP) CP, a 16.7% CP ration, composed of 7.0% UDIP and 9.7% DIP would be enough. To the best of our knowledge, no information has been published on the effect of more than 16.7% undegradable protein, during the first weeks after calving, on the cows' performance.

From information appearing in the literature, there is a definite trend showing that high proportions of DIP in dairy cow rations have a negative effect on their fertility (Kaim et al., 1983; Bruckental et al., 1989; Ferguson and Chalupa, 1989; Elrod, 1992). This is attributed mostly to blood urea level in the genital organs (Elrod et al., 1993). This hypothesis can be verified by supplying dairy rations with UDIP that does not lead to ammonia production in the rumen.

The objective of the present work was to determine the effect of providing supplemental UDIP to dairy cow rations above 16.7%, at different periods during lactation, on milk yield and composition and reproductive performance.

## MATERIALS AND METHODS

### *Animals and diets*

The experiment was carried out in the herd of Kibbutz Yavne on 156 Israeli-Holstein cows that were divided into two treatments: Control (i); dietary

CP concentration was 16.5%, of which 11.0% was rumen degradable (RDCP). High-protein (HP) (ii); dietary CP concentration was 17.8%; all the additional protein above 16.5% was planned to be undegradable. The ratio between EDOM and EDCP was 5:1, in both rations; the EDOM and EDCP contents of the two rations were also the same. Pairs of cows were prepared, similar in their lactation number, days from calving, milk yield of their last lactation and the amount of milk produced during the last month before the start of the experiment. The cows of each pair were assigned to the two treatments at random. Fifty-nine cows (29 cows in the control group and 30 cows in the high-protein group) were within 3 weeks after calving, and 23 cows (12 cows in the control group and 11 cows in the HP group) were 21-50 days post-calving when the experiment started.

The ingredients of the two rations and their chemical composition are described in Tables 1 and 2. The NEL concentration was the same for both rations.

TABLE 1  
Composition of experimental rations and effective degradabilities of crude protein (EDCP) and organic matter (EDOM) of the rations' ingredients

Ingredients	Control	High protein	EDCP	EDOM
	% in DM		% <sup>2</sup>	
Fish meal	—	0.6	48.0	45.3
Feather meal	—	0.6	31.8	40.4
Gluten meal	0.9	1.5	15.2	28.5
Rapeseed meal	2.6	1.8	50.3	50.5
Soyabean meal	1.2	1.2	44.4	54.3
Cottonseed meal	5.6	8.4	55.8	45.5
Whole cotton seeds	4.6	4.6	73.7	42.9
Wheat bran	11.6	7.1	77.8	57.9
Barley grain	6.3	7.0	83.9	78.0
Wheat grain	9.5	8.9	76.5	85.2
Corn grain	13.0	13.5	67.9	69.9
Tapioca	6.3	6.6	59.7	81.8
Corn cobs	7.4	7.4	75.0	55.0
Vetch hay	6.3	6.0	45.8	35.8
Wheat hay	2.1	2.2	72.2	43.1
Bean hay	2.1	2.4	63.5	46.0
Wheat silage	16.4	16.6	82.5	56.7
Fatty acid salts	0.6	0.3	—	—
Oil	0.1	0.1	—	—
NPN mix <sup>1</sup>	1.3	1.1	83.8	72.8
Vitamin and mineral mix	2.1	2.1	—	—

<sup>1</sup> urea: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>:ground milo:oil, 40:10:49:1

<sup>2</sup> percent of total CP or total OM

TABLE 2  
Chemical composition of experimental rations, % in DM

Component	Control	High protein
NDF	34.6	33.2
ADF	18.6	18.6
Crude protein	16.5	17.8
Degradable CP	11.0	11.0
Undegradable CP	5.5	6.8
Degradable OM	55.7	55.1
DOM : DCP <sup>1</sup>	5.05	5.03
NEL (Mcal/kg)	1.72	1.72
Calculated abomasal amino acid flow, g/d <sup>2</sup> :		
Histidine	77.8	83.7
Lysine	211.5	221.3
Methionine	61.8	67.6

NDF = neutral detergent fibre, ADF = acid detergent fibre

<sup>1</sup> DCP = Degradable CP, DOM = Degradable OM

<sup>2</sup> calculated according to Tagari et al. (1994)

### Management and reproduction

The experiment began after 10 days of adaptation to the different rations and lasted 75 days. The feed and refusals were weighed daily and sampled twice a week for chemical analysis. Daily milk yield was recorded every 2 weeks and analysed for its lactose, protein, fat and urea contents. Body scoring was carried out every 3 weeks. Two weeks prior to the end of the experiment, blood and rumen fluid samples were taken before the first morning meal and 3.5 h later. Samples were analysed for ammonia and volatile fatty acids (VFA) in the rumen fluid, and for urea in the blood.

During the first month following parturition, the cows were under intensive veterinary supervision with special emphasis on the status of the reproductive system. Visual observations of oestrus were carried out three times daily, commencing 45 days after parturition. Cows that were detected in oestrus were inseminated starting 45 days after calving, once each day. Cows were diagnosed for pregnancy by rectal palpation 45 to 55 days following insemination. The proportion of cows diagnosed as pregnant by three inseminations or fewer out of the total number of cows inseminated, was defined as pregnancy rate.

### Analytical methods

The chemical composition of ration ingredients was determined according to the AOAC (1980). *In-situ* degradabilities of organic matter (OM) and CP were

determined for all ingredients of the experimental rations by the dacron-sac technique (Ørskov and McDonald, 1979). Rations' EDOM and EDCP and the ratio between them were arranged by linear programming as described by Arieli et al. (1989). Ammonia-N and VFA in rumen fluid and blood urea-N were determined by the methods described by Tagari (1969).

### *Calculations and statistical analysis*

Data were analysed using the GLM procedure of SAS (1987). The model used was as follows:  $Y_{ijt} = (\mu + \alpha_j t + \beta \log(t) + Z_i + \delta Z_i t + e_{ijt})$ , where  $Y$  represents the yield (milk, fat or protein) of cow  $i$  allocated to treatment  $j$  at day  $t$  after calving;  $\mu$  is the intercept;  $Z_i$  is the previous 305-day milk yield of cow  $i$ ; the coefficients  $\alpha_j$ ,  $\beta$ , and  $\delta$  are to be estimated from the model and only  $\alpha_j$  is dependent on the treatments;  $e_{ijt}$  is the error term. Results were analysed for all the cows and separately for the cows that started the experiment immediately after calving. Fertility results are presented only for cows that entered the experiment within 2 weeks after calving. Means of treatments for reproductive criteria were compared by the chi-square method. Differences between treatments were deemed significant when  $P \leq 0.05$ . Body condition score data were calculated only for the cows that started the experiment within 2 weeks after calving. The amino acids composition of digesta leaving the rumen was predicted as described by Tagari et al. (1994).

## RESULTS

### *Performance of all cows*

The average daily dry matter intake (DMI) of the control and HP treatments was 21.2 and 20.7 kg, respectively (Table 3). Because the cows were group fed, it could not be determined whether the difference in feed intake between the experimental treatments was statistically significant. However, it is worth mentioning because these results are the average feed and refusals weights of each day during 75 days.

The HP cows consumed 200 g CP or 246 g UDIP more than the control cows. Their milk, milk fat, protein and 3.5% fat-corrected milk (FCM) yields (kg/d), and milk fat concentration (%) were higher by 1.2, 0.069, 0.039, 1.2 kg/d and 0.13%, respectively, than those of the control cows ( $P \leq 0.05$ ; Table 3).

TABLE 3  
Average daily dry matter intake and least square means of milk and yield of milk components of all cows, during 75 days of experiment (83 cows in each group)

	Control	High protein	SEM
No. of cows	83	83	
Dry matter intake, kg	21.2	20.7	
Crude protein intake, kg	3.50	3.70	
Milk, kg	34.3 <sup>a</sup>	35.5 <sup>b</sup>	0.41
Milk fat, %	2.54 <sup>a</sup>	2.67 <sup>b</sup>	0.039
Milk protein, %	2.85	2.85	0.015
Milk lactose, %	4.46	4.45	0.016
Milk fat, kg	0.866 <sup>a</sup>	0.935 <sup>b</sup>	0.017
Milk protein, kg	0.963 <sup>a</sup>	1.002 <sup>b</sup>	0.011
Milk lactose, kg	1.539 <sup>a</sup>	1.579 <sup>b</sup>	0.022
3.5% FCM, kg	31.3 <sup>a</sup>	32.5 <sup>b</sup>	0.41
Crude protein efficiency <sup>1</sup> , %	27.5	27.1	

<sup>1</sup> (milk crude protein/feed crude protein) x 100

<sup>a, b</sup> within rows, means not sharing a common superscript differ significantly ( $P \leq 0.05$ )

### Performance of cows that started the experiment after calving

For the cows that were immediately or within 0-21 or 22-29 d after calving, the average milk, milk fat and protein yields of the HP cows during the experimental period were higher than those of the control cows ( $P \leq 0.05$ ; Table 4). No difference between treatments could be shown for cows which started the experiment 30-44 days post-calving.

TABLE 4  
Least square means of average milk, milk fat and protein yields of cows that were given the experimental rations for at least 28 d, beginning at 0, 22 and 30 days post-calving

	Days after calving					
	0-21		22-29		30-44	
	Control	HP	Control	HP	Control	HP
	kg/d					
n	29	30	6	4	6	7
Milk	37.0 <sup>b</sup>	39.6 <sup>a</sup>	35.3 <sup>b</sup>	43.8 <sup>a</sup>	46.6	43.9
Milk fat	0.992	1.043	0.982 <sup>b</sup>	1.212 <sup>a</sup>	1.132	1.099
Milk protein	0.985 <sup>b</sup>	1.056 <sup>a</sup>	0.984 <sup>b</sup>	1.208 <sup>a</sup>	1.186	1.202

<sup>a, b</sup> within rows, means not sharing a common superscript differ significantly ( $P \leq 0.05$ )

Average body condition score (BCS) of the control cows which started the experiment immediately after calving, decreased throughout the experimental period and was lower at the end by 0.64 BCS units. Average BCS of the HP cows decreased up to day 50, after which these cows began gaining weight; by the end of the experiment they had gained 0.4 BCS units ( $P \leq 0.05$ ).

#### *Metabolites in rumen fluid and blood*

Ammonia-N and VFA levels in ruminal fluid and urea-N level in blood, before the morning meal and 3.5 h after it, are shown in Table 5. There was no significant difference in ammonia-N level in ruminal fluid between the control and the HP cows before the morning meal. During 3.5 h post-feeding, ammonia-N level in ruminal fluid of the control and HP cows increased by 5.7 and 5.9 mg/100 ml ( $P \leq 0.05$ ), respectively. No significant difference could be found between the control and HP cows in blood urea-N level before the morning meal and 3.5 h after it.

Total and individual VFA levels in rumen fluid (mMol/l) increased markedly during the first 3.5 h after the morning meal was given ( $P \leq 0.05$ ), but no difference was found between treatments in their levels before the morning meal and 3.5 h later. The only significant differences between treatments were lower C2:C3 and (C2 + C4):C3 ratios, 3.5 h after the morning meal, of the HP cows in comparison with the control cows.

TABLE 5

Rumen fluid ammonia-N and blood urea-N levels (mg per 100 ml), and total and individual volatile fatty acids (VFA) levels in rumen fluid (mMol), before the morning meal and 3.5 h after it

Time after meal, h	Control		High protein		SEM
	0	3.5	0	3.5	
	mg per 100 ml				
Ammonia-N	11.9 <sup>a</sup>	17.6 <sup>b</sup>	10.3 <sup>a</sup>	16.2 <sup>b</sup>	0.97
Urea-N	14.1 <sup>a</sup>	14.8 <sup>ab</sup>	15.2 <sup>ab</sup>	16.9 <sup>b</sup>	0.61
	mMol				
Total VFA	53.0 <sup>a</sup>	74.7 <sup>b</sup>	55.7 <sup>a</sup>	73.8 <sup>b</sup>	<b>3.00</b>
Acetic acid	33.8 <sup>a</sup>	43.7 <sup>b</sup>	34.8 <sup>a</sup>	42.1 <sup>b</sup>	<b>1.54</b>
Propionic acid	12.7 <sup>a</sup>	19.5 <sup>b</sup>	14.3 <sup>a</sup>	22.4 <sup>b</sup>	1.12
Butyric acid	5.2 <sup>a</sup>	8.9 <sup>b</sup>	5.0 <sup>a</sup>	7.8 <sup>b</sup>	0.47
Isocaproic acid	0.6	0.6	0.7	0.9	0.11
Caproic acid	1.0	1.9	0.8	1.6	0.14
Acetate/propionate	2.7 <sup>a</sup>	2.3 <sup>b</sup>	2.5 <sup>ab</sup>	1.9 <sup>c</sup>	0.09
Acet. + but./propionate	3.1 <sup>a</sup>	2.8 <sup>a</sup>	2.8 <sup>a</sup>	2.3 <sup>b</sup>	0.12

<sup>a, b</sup> within rows, means not sharing a common superscript differ significantly ( $P \leq 0.05$ )

*Reproductive performance*

In the year prior to the experiment, the reproductive performance of the cows in both treatments was similar (Table 6). Supplementation of additional undegradable CP at the beginning of lactation tended to improve reproductive performance: more cows conceived by three inseminations in the HP group (86 vs 68%) and fewer inseminations per conception were required (2.38 vs 2.68) than in the control group. More cows were culled because of fertility reasons in the control group than in the HP group (9 vs 1;  $P < 0.068$ ).

TABLE 6  
Reproduction performance during pre-experimental and experimental years, of cows that were given the experimental feeds for at least 2 weeks during the first period after calving

Treatment	n	Empty days	Insemination per conception	Pregnancy rate <sup>1</sup> (%)	Culled cows <sup>2</sup>
Pre-experimental year:					
Control	23	119.0	2.61	78	–
High-protein	21	126.5	2.62	67	–
SEM		11.1	0.30		
Experimental year:					
Control	23	102.8	2.68	68	9 <sup>a</sup>
High-protein	21	124.0	2.38	86	1 <sup>b</sup>
SEM		10.2	0.25		

<sup>1</sup> cows conceived by 3 inseminations or less out of the total number of cows inseminated

<sup>2</sup> cows culled from fertility reasons only, due to veterinarian followed-up inspection and decision

<sup>a, b</sup> significantly different ( $P \leq 0.05$ )

## DISCUSSION

*All cows performance*

The DM intake of the cows that were given additional UDIP tended to be lower than that of the control cows (Table 3). This finding agrees with other studies that reported lower DMI as a result of increasing dietary CP level, especially when low-degradable protein, like fish meal, was supplied (Bruckental et al., 1989; Newbold and Rust, 1990; Tomlinson et al., 1990). In the present study, the increase in the CP and UDIP levels of the HP ration was achieved by changes in the amount of the control ration protein supplements, in order to avoid basic differences between the rations (Table 1). Likewise, a relatively small amount of fish meal (0.6% DM) was added to the HP ration. It seems that the influence of low-degradable CP level in the diet on DMI cannot be attributed



always to the negative effect of a specific protein supplement (Newbold and Rust, 1990). A diversity of dietary protein supplements and an improvement in the amino acid profile of protein reaching the abomasum (Table 2; Titgemeyer et al., 1989; Tagari et al., 1994) and absorbed from the intestine, might improve feed energy and protein utilization and reduce dietary energy requirements (Dror et al., 1970; Klopfenstein et al., 1982).

Experimental rations were calculated to supply similar amounts of EDOM and EDCP and a similar EDOM to EDCP ratio of 5:1. Accordingly, no differences in fermentation parameters between treatments were expected. Total VFA concentration in rumen fluid and individual VFA concentrations before the morning meal and 3.5 h later were similar in the two treatments (Table 6). In a previous study (Bruckental et al., 1989), when fish meal was the only supplemental CP in the milking cows' ration, a decrease in total VFA concentration in rumen fluid was found, before the morning meal and 3 h after it, in comparison with cows fed rations supplemented with soyabean meal or the low-CP control ration. Similar results were obtained by Newbold and Rust (1990). Likewise, no statistical difference between treatments in the average rumen ammonia-N and blood urea-N concentrations was found (Table 6). This indicates that dietary UDIP could be increased without affecting microbial fermentation in the rumen and that the additional UDIP was utilized efficiently.

#### *Post-calving cows performance*

A ration containing 1.67 Mcal NEL and 19% CP per kg DM, is recommended by the NRC (1989) for cows during the first 3 weeks after calving. However, when CP is incorporated in the diet at a DIP:UDIP ratio of 58:42, a 16.7% CP ration could supply all protein needs. Later on, it is recommended to elevate NEL concentration up to 1.72 Mcal per kg DM, which enables the increase in DIP to 63%, without changing the dietary CP level. In the present study, the control ration consisted of 1.71 Mcal NEL/kg DM and 16.5% CP, of which 67% was DIP (Table 2). Elevation of the dietary CP level by 1.3% (all of which was UDIP) during the first 4 weeks after calving, improved milk, milk fat and protein yields (Table 4). Cows that were given the HP diet starting 30 days after calving or later, did not respond with higher milk production. Accordingly, the data of the present study agree with NRC (1989) recommendations that feeding management of cows during the first 3 weeks after calving should be different with respect of CP supply, than later in lactation. However, our data prove that increasing dietary CP level above 16.5% with 1.3% UDIP, was required for the earlier recovery of body condition and for the increase in milk, milk fat and protein yields during the experimental period (Table 4), as compared with the control cows.

Reproductive performance of the HP cows tended to be better than that of the control cows: fewer inseminations per conception were needed, a higher conception rate was achieved and only one cow was culled due to fertility problems, in comparison with nine cows culled in the control group ( $P \leq 0.05$ ; Table 6). Many studies pointed to the fact that overfeeding of protein may be associated with a decline in fertility (Jordan and Swanson, 1979; Kaim et al., 1983; Bruckental et al., 1989; Canfield et al., 1990). Elrod et al. (1993) concluded that excess DIP, regardless of source or degradability, significantly alters uterine pH, and this may play a role in the observed reduction of fertility. Data presented in the present study confirm that dietary CP concentration could be raised without affecting blood urea level (Table 5). Provided that the dietary ratio of EDOM:EDCP is not altered in the HP ration as compared with the lower CP ration, a maximal capture of soluble ammonia-N in rumen fluids and minimal escape of it into portal blood might occur (Bruckental et al., 1986). Under such conditions, when dietary CP is elevated by the addition of undegradable protein only, no reduction in fertility is expected. Butler and Smith (1989) claim that the post-partum deficiency of protein probably has an immediate effect on the recovery of the cow and a long-term negative effect on the genital organs' function. Additional supply of UDIP in the present study might be responsible for the earlier recovery of body condition score of the HP cows and the better reproductive performance as compared with the control cows.

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## STRESZCZENIE

**Wpływ uzupełnienia dawek białkiem nierozkładalnym w żwaczu na produkcję i skład mleka oraz reprodukcję krów we wczesnym okresie laktacji**

W doświadczeniu przeprowadzonym w układzie ciągłym sto pięćdziesiąt sześć krów Holsztynów Izraelskich podzielono losowo na dwie grupy; 59 krów weszło do doświadczenia, trwającego 75 dni, w 14 dniu po wycieleniu. Dawka 1 (grupa kontrolna) zawierała 16,5% białka ogólnego (b.og.), z czego 11,0% białka rozkładalnego w żwaczu (RDCP). Dawka 2, wysokobiałkowa (HP) zawierała 17,8% b.og.; całe dodatkowe białko było nierozkładalne w żwaczu. Zwierzęta żywiono grupowo. Ilość pobranej paszy i produkcję mleka oznaczano codziennie, skład mleka – co 2 tygodnie. Pobranie suchej masy, produkcja mleka, tłuszczu i białka mleka (kg/d) w grupie kontrolnej i wysokobiałkowej wynosiły 21,2 i 20,7 ( $P \leq 0,05$ ); 34,3 i 35,5 ( $P \leq 0,05$ ); 0,866 i 0,935 ( $P \leq 0,05$ ); oraz 0,963 i 1,002 ( $P \leq 0,05$ ), odpowiednio. Średnia produkcja mleka, tłuszczu i białka mleka krów, wchodzących do doświadczenia między 0 a 21 dniem po wycieleniu, grupy kontrolnej i wysokobiałkowej wynosiła 37,0 i 39,6 ( $P \leq 0,05$ ); 0,992 i 1,043; oraz 0,985 i 1,056 ( $P \leq 0,05$ ) kg/d, odpowiednio. Przy uzupełnieniu dawki dla krów w początkowym okresie laktacji dodatkową ilością białka nierozkładalnego w żwaczu stwierdzono tendencję poprawy użyteczności rozplodowej, co przypisuje się wcześniejszej poprawie kondycji tych krów.