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EFFECT OF FEEDING DIFFERENT CRUDE PROTEIN DIETS ON REPRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COW

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ABSTRACT

In light of increasing global protein prices and with the need to reduce environmental impact of contemporary systems of milk production, the current study was performed to evaluate the effect of feeding on ration with different crude protein CP levels, in Holstein dairy cows ration on feed intake, milk production, reproductive performance and some hormonal and biochemical parameters in blood serum and milk. Two hundred and four Holstein dairy cows in three groups, 103, 63 and 38 were fed diets containing 20% CP, 18.4% CP and 17.5% CP for Groups 1, 2 and 3, respectively. Amino acid methionine and rumen undegredable protein (RUP) increased by decreasing the amount of CP. The average feed consumption and average milk production /head/day was reported. Gonadotrophin, Prostaglandin, Gonadotrophin protocol "GPG" were applied to the studied groups and artificial insemination were applied and ultrasonography was used to detect pregnancy and different reproductive findings. Blood and milk samples were collected to estimate serum progesterone hormone and milk urea nitrogen (MUN). Milk from each group was collected for analysis of milk compositions. The obtained results showed non-significant difference in the average dry matter intake per head per day between the studied groups (25.65 ± 0.92 kg), (24.45 ± 0.60 kg) and (24.62 ± 0.49 kg) respectively, with significant increase ($P < 0.05$) in the average milk production /head/day in group 3 (35.34 ± 1.27 kg) compared to group 1 (31.63 ± 0.57 kg). The analysis of milk samples revealed no significant difference in its constituents except in case of fat % which showed significant increase ($P < 0.05$) in group 3, (3.94 ± 0.04 %) compared with group 2, (3.70 ± 0.05) also milk samples showed significant decrease ($P < 0.05$) in the average milk urea nitrogen concentration in group 3 (13.09 ± 1.15 mg/dl) compared to group 1 (16.64 ± 0.79 mg/dl). The obtained results showed highly significant increase ($P < 0.01$) in conception rate in groups 3 and 2 (39.47%) and (41.27%) respectively compared with group 1(17.48%). Serum progesterone level 12 days post-insemination showed significant increase ($P < 0.05$) in group 3 (2.06 ± 0.25 ng/ml) compared to group 1 (0.74 ± 0.11 ng/ml). This study concluded that reducing the CP level as in group 3 with adjusting the requirements of amino acids, energy and RUP improved fertility and profitability in Holstein dairy cows.

Key words: *Crude protein, Dairy farms, Progesterone hormone, Milk urea nitrogen, Conception rate, Holstein dairy cows.*

INTRODUCTION

All of the newer dynamic models for balancing rations for cows no longer use the older more ill-defined parameter of crude protein (CP). Rather, they try to balance for what the cow truly needs, which is metabolizable protein (MP). Feeding diets with lowered protein content reduces nitrogen input, improves nitrogen utilization efficiency and reduces nitrogen losses from manure (Sinclair, 2012). Reducing dietary protein also benefits the producer by reducing feed cost and improving overall farm profitability Lee *et al.* (2011). Dry matter intake (DMI) was decreased when feeding the metabolizable protein (MP) deficient diets, milk production also decreased Lee *et al.* (2012). Protein can increase milk yield by providing more amino acids, by increasing available energy and by altering efficiency of utilization of absorbed nutrients Yasothai (2014).

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There are two sources of MP available for the cow; first is the true protein in the diet that escapes degradation in the rumen; this is called rumen undegradable protein RUP and the second source is the bacterial protein produced by the rumen microbes. Rumen undegradable protein seems to receive the most attention by many nutritionists; the average amino acid composition of microbial protein is similar to the composition of milk (Tamminga, 2006). Typically, protein-rich feed stuffs have much greater dissimilarities in amino acid profiles for their RUP when compared to milk protein. The balancing diets for amino acids are reasonably beneficial and more accurate and make the need for a high level of CP is not necessary Block (2006). Protein requirements are calculated according to animal body weight, milk production and physiological state. However, due to individual animal variation, accurate prediction of animal protein requirements is difficult. Therefore, in order to maintain high milk production, most of the high-producing cows are fed protein at greater level than recommended, thus resulting in elevated urea concentrations (Ferguson *et al.*, 1993).

Feeding excess rumen degradable protein RDP has a negative effect on fertility delaying the first ovulation or oestrus, reducing the conception rate of first insemination, increasing the days open and lowering the overall conception rate (Tamminga, 2006). There are several proposed mechanisms for this effect including an exacerbated negative energy balance NEB for cows fed diets high in rumen degradable protein RDP in comparison to diets high in RUP (Westwood *et al.,* 2000) and proven deleterious effects of both ammonia and urea on both oocyte and embryo development (Rhoads *et al.,* 2006). Zanton *et al.* (2014) stated that, feeding rumen-protected methionine increased milk protein percent, milk fat percent and milk yield. Nikkhah *et al.* (2013) found that, cows receiving supplemental methionine experienced an increase in dry matter intake, milk yield, and fat and protein percent; there was also improvement in several aspects of reproductive function, including a reduction in days to estrus and conception.

The aim of this study was to evaluate the effect of feeding different crudeprotein diets with adjusting the requirements of amino acids and RUP on reproductive performance of Holstein dairy cows in the Egyptian farm circumstances.

MATERIALS AND METHODS

Two hundred and four Holstein dairy cows from Al-yoser dairy farm in Ismailia with average DMI (180 d) were utilized in a completely randomized research design to study the effects of feeding different crude protein diets with different RUP and adjusting the essential amino acid of MP on milk production and reproductive responses to a scheme of estrus synchronization, conception rate and determine the feed cost. Cows were divided into three groups, the 1st group: 103 cows fed diet containing 20% CP with 36.87% RUP, the 2nd group: 63 cows fed diet containing 18.4% CP with 41.3% RUP, while the 3rd group: 38 cows fed diet containing 17.5% CP with 45.71% RUP. The three groups were fed isocaloric diets in the same farm, season (winter), and have the same management and Dietary treatments were initiated 35 d prior to the beginning of the estrus synchronization scheme. The experimental diets were formulated to meet all the nutritional requirements according to (NRC 2001). Cows were fed diets for ad libitum consumption and given ad libitum access to water. Cows were milked three times a day and milk per cow was recorded. All cows were high producing ones and were more than 60 days postpartum and exposed to ovisynch. Estrus synchronization scheme (Gonadotrophin, Prostaglandin, Gonadotrophin protocol “GPG”), all cows were free from any reproductive disorders before artificial insemination and only cows showed estrus with ovulation were inseminated. Cows suffered from lameness and mastitis was registered. The cows had a response to estrus synchronization were artificially inseminated, cows showed estrus after AI and before ultrasound scanning were calculated and others were diagnosed for pregnancy and reproductive problems at day 27 post insemination. The pregnant cows confirmed for pregnancy at day 40 post insemination using ultrasound scanning and the number of cows showed early embryonic death was recorded.

Table 1: Feed composition and proximate analysis of the experimented diets.

Feed Stuff (Kg/h/d)	R1 (20.3)	R2 (18)	R3 (17.5)
Corn Grain, Ground , Dry	5.25	5.25	5.25
High fat soya	3	3	3
Soybean, Meal, Solv, 47% protein	2.5	1.75	-
Wheat bran	0.5	0.5	0.5
Corn, Dry, Distiller Grain (DDGS)	-	-	1.75
Cotton seed, Whole with lint	2	2	2
Calcium soaps of fatty acids	0.3	0.3	0.3
Corn silage	15	15	15
Alfa Alfa hay	5.2	2.2	2.2
Egyptian clover (barseem)	-	18	18
Magnesium oxide	0.04	0.04	0.04
Sodium Bicarbonate	0.16	0.16	0.16
Mono- basic calcium phosphate	0.08	0.08	0.08
Premix[1]	0.06	0.06	0.06
NEW T-NIL®Dry[2]	0.02	0.02	0.02
MTB 100	0.01	0.01	0.01
Crude Protein CP	20	18.4	17.5
Net Energy for lactation NEL	1.76	1.76	1.76
NDF	29.6	29.4	30.9
ADF	20.1	19.3	19.9
R/C Ratio	40.7	40.7	40.7
TDN	75	75	75
Ether Extract E.E	6.2	6.8	7
DM	61.6	42.00	42.86
RUP (% of CP)	36.87	41.3	45.71
Lysine AA (Percent of MP)	6.63	6.5	6.27
Methionine AA (Percent of MP)	1.84	1.86	1.9
Lysine/ Methionine ratio	3.6	3.5	3.3
Ration cost U.S ($) (100% As-fed)	8.92	8.42	8.05
Feed cost, $/cow/day	10.88	10.13	9.56

According to the official methods of A.O.A.C. (2012).

1= (Vit. A 10000000 IU, Vit D3 2500000 IU, Vit. E 35000 mg, Biotin 1000 mg, Zinc 100000 mg, Mn 80000 mg, Cu 30000 mg, I 800 mg, Co 400 mg, Se 300 mg, Caco3 to 3 kg). 2=Sorbic acid 0.05%, Citric acid 0.75%, Calcium propionate 10.5%, Copper sulphate 5%, Inactivated yeast (Saccharomyces Cerevisiae) 2%, Sapiolite 41.7%, Bentonite 40%.

Experimental measurements and laboratory analysis:

BW and feed consumption

Nutrients requirements were calculated on body weight basis and milk yield according to (NRC, 2001). Amounts of ration fed and feed refusals were recorded daily for each group and the average daily DM intake and cost for each ration of each group was calculated.

Analysis of dietary nutrients content:
Dry samples from the three rations were ground with a Wiley mill (2-mm screen). Feed samples were analyzed for ether extract and Crude protein according to A.O.A.C. (2012). NDF and ADF were determined according to Goering and Van Soest (1991).

Milk production and collection:
Individual milk yield was recorded daily two weeks after the feeding trial had begun till its end. Milk samples were collected from each group every (15 days) and analyzed for MUN, fat, protein, solids-not-fat using Milko-Scan FT 6000).

Blood collection:
Blood samples were taken from randomly selected 10 dairy cows from each group through jugular vein puncture 12 day post insemination.

Samples were collected in a clean centrifuge tube and were allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non-hemolized supernatant serum was harvested and frozen at -20 c^o for progesterone analysis using the method of ELIZA According to Tietz (1995).

Statistical Analyses:
The various data were subjected to ANOVA procedure for a randomized complete design, Analysis of variance by Duncan's test according to Snedecor and Cochran, (1982). Least significant difference was applied to the data to test for differences between treatments using a computer program 'SPSS', Significance was declared at ($P < 0.05$). Qi square was performed to the reproductive parameters of the studied groups.

RESULTS

The obtained results showed a non-significant difference in the average DMI per head per day in the three studied groups (25.65 ± 0.92), (24.45 ± 0.60) and (24.62 ± 0.49) for group (1), (2) and (3) respectively, as seen in table (2).

Table 2: Average feed consumption (DMI) / head/day expressed by Kg (mean ± SE).

CP content in ration	Group (1)	Group (2)	Group (3)
Average consumption /head/day (Kg)	25.65 ± 0.92	24.45 ±0.60	24.62 ± 0.49

The results showed a significant increase (P < 0.05) in the average milk production (35.34 ± 1.27 kg/head/ day) in group (3) compared to group (1) (31.63 ± 0.57) as seen in table (3).

Table 3: Average milk production / head / day expressed by Litre (mean ± SE).

Cp content in ration	Group (1)	Group (2)	Group (3)
Average milk production /head/day (L)	31.63 ± 0.57^{a}	33.77 ± 0.72^{ab}	35.34 ± 1.27^{b}

a,b Means in the same row with different superscripts differ, ($P < 0.05$).

Analysis of milk for the three studied groups showed a significant increase (P < 0.05) in fat % in group (3), (3.94 ± 0.04) compared with group (2), and significant decrease (P < 0.05) in the average of Milk urea nitrogen MUN concentrations in the group (3), (13.09 ± 1.15) compared to group (1), (16.64 ± 0.79) as seen in table (4).

Table 4: Analysis of milk samples for studied groups (mean ± SE).

Groups	Group (1)	Group (2)	Group (3)
Fat %	3.82 ± 0.05^{ab}	3.70 ± 0.05^{b}	3.94 ± 0.04^{a}
Protein %	3.250 ± 0.034^{a}	3.17 ± 0.033^{a}	3.16 ± 0.03^{a}
SNF	8.917 ± 0.031^{a}	8.87 ± 0.021^{a}	8.88 ± 0.037^{a}
Milk Urea Nitrogen (MUN)	16.64 ± 0.79^{a}	15.35 ± 0.88^{ab}	13.09 ± 1.15^{b}

a,b Means in the same row with different superscripts differ, (P < 0.05).

Highly significant increase (P < 0.01) in conception rate in groups (2) and (3) compared to group (1), the percentage of lameness and mastitis showed non significant differences between groups, as seen in table (5).

Table 5: Reproductive parameters in the studied groups.

	Total cows	Cows show estrus before ultrasound exam	Sonar Exam		Conception rate	Cows showed lameness	Cows showed mastitis
			Pregnant	non pregnant			
Group (1)	103	49	18 (17.48)	36 (34.95)	17.48 % [a]	5(4.85%)	1(0.97%)
Group (2)	63	27	26 (41.27)	10 (15.87)	41.27 %[b]	3 (8.33%)	4(6.35%)
Group (3)	38	16	15 (39.47)	7 (18.42)	39.47% [b]	0 (0%)	1(2.63%)

a,b Means in the same row with different superscripts differ, (*P* < 0.01).

The results showed non significant differences in the studied reproductive findings (cyst, follicles, corpus lutium and dead foeti) between group (1), (2) and (3). As seen in table (6).

Table 6: Reproductive findings in the studied groups (non-pregnant cows scanned with ultrasound).

Group no.	Sonar Exam			
	Cyst	Follicles	CL	Dead Foeti
Group (1)	**8 (7.77 %)[a]**	**2 (1.94 %)[a]**	**20 (19.42 %)[a]**	**2 (1.94 %)[a]**
Group (2)	**3 (4.76 %)[a]**	**0 (0 %)[a]**	**6 (9.52 %)[a]**	**4 (6.35)[a]**
Group (3)	**3 (7.89 %)[a]**	**0 (0 %)[a]**	**3 (7.89 %)[a]**	**0 (0 %)[a]**

a,b Means in the same row with different superscripts differ, P < 0.05.

The obtained results also showed significant increase (P < 0.05) in progesterone hormone levels in group 3 (2.06 ± 0.25) compared to group 1 (0.74 ± 0.11) ng/ml, (table 7).

Table 7: The average serum progesterone concentration in the studied groups (mean ± SE).

Parameters	Group (1)	Group (2)	Group (3)
Progesterone ng/ml	0.74±0.11[b]	1.19±0.23 [ab]	2.06±0.25[a]

a,b Means in the same row with different superscripts differ, P < 0.05.

DISCUSSION

The results showed non-significant difference in the average of DMI per head per day in the three studied groups (25.65 ± 0.92), (24.45 ± 0.60) and (24.62 ± 0.49) for groups (1), (2) and (3) respectively, as seen in table (2) these results coincide with that reported by Nadeau *et al.* (2007) who concluded that rations with 16 to 17% CP were adequate for dairy cows when diets were balanced including rumen degradable (RDP) and undegradable (RUP) proteins. Also, Van Amburgh *et al.* (2007) and Higgs (2009) found that reduced crude protein CP rations can be fed in commercial dairy herds and support high milk production.

The results showed a significant increase (P < 0.05) in the average milk production (35.34 ± 1.27 kg/head/day) for group (3) compared to group (1) (31.63 ± 0.57), these results agreed with Robinson (2010), this increase in milk production can be explained as the over-feeding of rumen degradable protein RDP in group (1) to the point that rumen ammonia concentrations markedly exceed bacterial requirements and MUN become high. Not only does it result in wastage of RDP, but there is also good evidence that it decreases flows of microbial protein to the small intestine (Boucher *et al.*, 2007). This can have significant implications on both farm profitability and nutrient management practices. So the significant increase in the average milk production in group (3) may be due to the higher level of RUP % and adequate percentage between essential AA lysine to methionine in group (3) compared to group (1). In addition, Dairy cattle do not have a CP requirement but do need absorbable amino acids to meet requirements to support lactation, pregnancy, maintenance and growth. A more biologically correct way to balance rations is using the MP approach. This is outlined in more detail by Varga (2007). The increase in milk yield level in group (3) may be a response to lysine/ methionine ratio adjustment Robinson (2010). The positive effects of feeding cows adequate amount of important essential amino acids like methionine in low protein diet with a high level of RUP is more beneficial than feeding a high total CP diet Nikkhah *et al.* (2013).

Analysis of milk for the three studied groups showed a significant increase ($P < 0.05$) in fat % in group (3), (3.94± 0.04) compared with group (2), and significant decrease ($P < 0.05$) in the average of Milk urea nitrogen MUN concentrations in the group (3), (13.09± 1.15) compared to group (1), (16.64 ± 0.79) as seen in table (4). These results agreed with Broderick and Clayton, (1997) and Sawa *et al.* (2011), who found that, excessive feeding of protein can lead to increased MUN concentrations. Also, Lee *et al.* (2012) who recorded significant improvements in milk yield relative to amino acid supplementation. Tables (5)& (6) showed highly significant increase ($P<0.01$) in conception rate in group (2) and group (3) compared to group (1), and these results were not affected by number of cows showed lameness or mastitis in each group although, non significant differences were showed in other reproductive findings (cyst, follicles, corpus lutium and dead foeti) between groups (1), (2) and (3), these results were in agreement with Lean *et al.* (2012) and Albaaj *et al.* (2017) whom reported that, Dietary protein levels are a risk factor for poor reproductive performance and found that, Conception is particularly impaired in cases of high blood or milk urea (Butler, 2001). Lean *et al.* (2012) reported an overall 9% reduction in conception in cows fed diets containing higher or more degradable CP within a dietary CP range of 111 to 230g/kg. Aboozar *et al.* (2012) reported improvements in a range of reproductive traits in cows fed high levels of rumen undegradable protein.

The obtained results also showed significant increase ($P < 0.05$) in progesterone hormone levels in group 3 (2.06 ± 0.25) compared to group 1 (0.74 ± 0.11) ng/ml (table 7). These results agreed with those reported by (Carroll *et al.*, 1988; Barton *et al.*, 1996 and Jorritsma *et al.*, 2003) who recorded that, Excess dietary CP may inhibit fertility in some cases by lower plasma progesterone concentrations. Sinclair *et al.* (2000) found cows that fed high protein diets generate high rumen ammonia and resulted in elevated ammonia levels in follicular fluid result in Oocytes with lower cleavage rates compared to heifers feddiets generating low ammonia. The decline in fertility may be attributed to the combined effects of a uterine environment that is dependent on progesterone, but has been rendered suboptimal for embryo development by antecedent effects of negative energy balance and can be further compromised by the effects of urea or ammonia resulting from intake of high dietary protein (Butler, 2005) and Staples *et al.* (1998) whom recorded that the Elevated concentrations of urea in blood or milk have been associated with reduced reproductive performance of lactating dairy cows. Also, decreased progesterone concentration has been suggested as a cause of decreased fertility and early embryonic losses in dairy cows Senatore *et al.* (1996).

CONCLUSION

Reducing the CP level to 17.5 with adjusting the requirement of amino acids, energy and RUP increase the efficiency of using dietary protein, increase milk productivity, fertility and profitability in Holstein dairy farms and decrease the hazards of environmental polution.

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تاثير تغذية مستويات مختلفه من البروتين الخام على الاداء التناسلى فى ابقار الهولشتين الحلابه

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في ضوء ارتفاع أسعار البروتين العالمية ومع الحاجة إلى الحد من التأثير البيئي للنظم المعاصرة لإنتاج اللبن، أجريت هذه الدراسة لتقييم تأثير تغذية مستويات مختلفة من البروتين الخام في علائق الأبقار الهولشتاين على معدلات تناول العلف، وإنتاج الحليب، والأداء التناسلي وبعض العناصر الهرمونية والبيوكيميائية في مصل الدم والحليب. مائتان وأربعة من أبقار الهولشتاين الحلابة بهذه الدراسة في ثلاث مجموعات، ١٠٣ و ٦٣ و ٣٨ حصلت علي علائق تحتوي على ٢٠٪ و ١٨.٤٪ و ١٧.٥٪ بروتين خام للمجموعات ١ و ٢ و ٣ على التوالي، تم اضافة الحمض الأميني الميثيونين والبروتين الغير متحلل بالكرش مع خفض كمية البروتين الخام. تم متابعة متوسط استهلاك العلف ومتوسط إنتاج الألبان / الرأس / اليوم. تم تطبيق بروتوكول توحيد الشبق الجي بي جي على المجموعات المدروسة وتم التلقيح الاصطناعي واستخدام الموجات فوق الصوتية للكشف عن الحمل والنتائج التناسلية المختلفة وكذلك تم تجميع عينات الدم واللبن لتقدير هرمون البروجسترون في الدم واليوريا بكل مجموعة وتحليل مكونات اللبن للمجموعات محل الدراسة. اظهرت النتائج عدم وجود فرق معنوي في متوسط تناول المادة الجافة لكل رأس في اليوم بين المجموعات محل الدراسة (٢٥.٦٥ ± ٠.٩٢ كجم) و (٢٤.٤٥ ± ٠.٦٠ كجم) و (٢٤.٦٢ ± ٠.٤٩ كجم) على التوالي، مع زيادة معنوية (p< ٠.٠٥) في متوسط إنتاج اللبن / الرأس / اليوم في المجموعة الثالثة (٣٥.٣٤ ± ١.٢٧ كجم) مقارنة بالمجموعة ١ (٣١.٦٣ ± ٠.٥٧ كجم). اظهر تحليل عينات اللبن للمجموعات الثلاث عدم وجود فرق معنوي في مكوناتها الا في حالة نسبة الدهون التي أظهرت زيادة معنوية (p< ٠.٠٥) في المجموعة الثالثة (٣.٩٤ ± ٠.٠٤ %) مقارنة مع المجموعة الثانية (٣.٧٠ ± ٠.٠٥ %). كما اظهرت عينات اللبن انخفاضا معنويا (p< ٠.٠٥) في متوسط تركيز اليوريا في المجموعة الثالثة (١٣.٠٩ + ١.١٥) مقارنة بالمجموعة الاولي (١٦.٦٤ + ٠.٧٩). واظهرت النتائج زيادة معنوية (p< ٠.٠٠١) في معدل الحمل في المجموعة الثالثة (٣٩.٤٧ %) والثانية (٤١.٢٧ %) مقارنة مع المجموعة الاولي (١٧.٤٨ %). اظهر مستوي البروجسترون في مصل الدم ١٢ يوما بعد التلقيح زيادة معنوية (p< ٠.٠٥) في المجموعة الثالثة (٢.٠٦ + ٠.٢٥ نانوجرام/ مل) مقارنة بالمجموعة الاولي (٠.٧٤ + ٠.١١ نانوجرام/ مل). وخلصت هذه الدراسة الي ان خفض مستوي البروتين الخام في العليقة كما بالمجموعة الثالثة مع ضبط الاحماض الامينية والطاقة والروتين الغير متحلل بالكرش يؤدي لتحسين الخصوبة والربحية في الابقار الهولشتاين الحلابة.

الكلمات الدالة: البروتين الخام - مزارع الالبان - هرمون البروجيسترون – معدل اليوريا باللبن – معدل الاخصاب – ابقار الهولشتاين الحلابة.