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Variations in the Production, Qualitative Characteristics and Coagulation Parameters of the Milk of the Riverine Buffalo Determined by the Energy/Protein Content of the Diet

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ABSTRACT : Sixteen Mediterranean pluriparous buffaloes were subdivided into two uniform groups of eight animals. The average weight of the two groups at the start of the trial was 671.2 and 656.7 kg. The number of days from calving were 33.4 and 33.3, and the average milk production was 12.73 and 12.33 kg/d. The trial lasted for 114 days, and was divided into two sub-periods of 58 and 56 days. The two diets, administered *ad libitum*, had the same forage/concentrate ratio (53/47) but in their formulation the percentage of the two forages varied. Diet 1: alfalfa hay = 10%, maize silage = 43%, concentrate 1 = 47% (6.63 MJ/kg DM of net energy; 179.5 g/kg DM of crude protein). Diet 2: alfalfa hay = 20%, maize silage = 33%, concentrate 2 = 47%, (5.99 MJ/kg DM of net energy; 155.4 g/kg DM of crude protein). For the overall trial period (33-146 days in milk), the intake of dry matter was 17.23 kg/d for Group 1 and 17.29 kg/d for Group 2 and corresponded to 2.50 and 2.58% ($p<0.01$) of live weight. There was no significant difference between the average weight (689.7 and 669.4 kg) and the body condition score (6.49 and 6.42) of the two groups of buffaloes. Group 1 produced a greater quantity of milk (11.89 vs. 10.90 kg/d, $p<0.10$) of better quality both for its higher fat content (82.32 vs. 77.29 g/kg, $p<0.10$) and its protein content (47.36 and 46.38 g/kg). The milk produced by the buffaloes receiving Diet 1 had a better clotting ability, lower values of r (15.98 and 16.42 min) and K_{20} (1.66 and 1.75 min) and a higher value of A_{30} (54.45 and 52.73 mm). Taking into consideration the two sub-periods, milk production was significantly different only in the first sub-period (33-90 DIM), in favour of Group 1 (13.08 vs. 11.56 kg/d, $p<0.05$), while the positive effect of Diet 1 was cancelled out (10.71 and 10.24 kg/d) in the second part of the trial (91-146 DIM).
(**Key Words :** Buffalo, Intake Capacity, Quanti-qualitative Milk Parameters)

INTRODUCTION

Breeding of the Mediterranean buffalo (*Bubalus bubalis* L.) in Italy is undergoing continuous expansion, even outside the traditional areas located in the central and southern areas of the country. This phenomenon is principally determined by three factors: i) the milk quotas, set by the European Union, impose limits on the production of cows' milk; ii) the price of buffalo milk, which is used exclusively for transformation, is approximately three times higher than that for cows' milk; iii) the availability of mozzarella is inferior to the market demand, both nationally and internationally (USA, France, Germany, United Kingdom), and, according to de Stefano (1999), in order to satisfy this market demand it would be necessary to double the number of lactating buffaloes.

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Concurrently with the increase in numbers being bred, modern stabling and milking techniques have been developed. Feeding management has been difficult due to the fact that only a few years ago the nutritional requirements were unknown since these animals were free to range on open pasture or were fed principally with industrial by-products while today the same foods are used as for the dairy cow. Now the Italian buffalo dairy farming industry has achieved economic importance, there has at the same time been an interest in increasing medium-level production. Definition of the energy and protein allowances of the lactating buffalo has become of fundamental importance since, with the optimization of feeding rations in the different productive phases, there is a greater economic advantage for businesses as well as control over environmental pollution.

The appropriate protein level at the different stages of lactation is still not sufficiently validated since only a limited literature exists, which provides contrasting indications (Verna et al., 1994; Campanile, 1998). A recent study (Bartocci et al., 2006) has demonstrated that through the administration of a diet with a high protein and energy level, a positive effect on the quantitative production of milk is achieved. Puppo et al. (2002) have demonstrated similar digestibility of crude protein by the Friesian cow and the Mediterranean buffalo. A higher ammonia level was found in the rumen of the buffalo (Kennedy et al., 1992; Bittante et al., 1994), which is more voluminous than that of the cow (Bartocci et al., 1997) with a greater capacity to degrade both crude protein and protein-free dry matter (Terramoccia et al., 2000). Bartocci and Terramoccia (2006) found that, in the buffalo species, the digestibility of the undegraded protein is higher than that of microbial origin; Nisa et al. (2008) have demonstrated that high ruminally undegradable protein diet increased nutrient intake and milk yield.

Recent research studies (Di Lella, 1998; Bartocci et al., 2002; Infascelli, 2003; Bartocci et al., 2006) have also sought to define the intake capacity of the lactating buffalo, but with conflicting results.

The aims of this study were to evaluate the effect of increased protein level on utilization of two diets, with energy concentration similar to that of previous work (Bartocci et al., 2006), in order to investigate the intake of dry matter and quantitative and qualitative milk production as well as to define the energy/protein allowances necessary for medium-high production in the first stage of lactation.

MATERIALS AND METHODS

Animals and diets

Sixteen multiparous buffaloes were used in the trial, and were subdivided at the beginning of the study into two uniform groups of eight animals. The average production of the previous lactations was 2,147 and 2,388 kg, for 255 and 259 days; the number of lactations had been 2.66 and 3.16, and the distance from calving was 33.4 and 33.3 days. The average level of milk production of the two groups at the start of the trial, was 12.73 and 12.33 kg/d; the average weight was 671.2 and 656.7 kg. The animals were weighed monthly and at the end of the trial. The body condition score (BCS) of the two groups of buffaloes at the beginning of the experiment was 6.24 and 6.23; the degree of fattening was determined monthly by three estimators according to the method of Wagner et al. (1988) as modified by Campanile et al. (1998) for the lactating buffalo. This method provides for the use of a score which ranges from 0-9. The differences between the two groups, for all the above-mentioned parameters, were not significant. The trial lasted 114 days (33-146 days in milk, DIM) and was subdivided into two sub-periods of 58 (33-90 DIM) and 56 days (91-146 DIM). There were two milkings, with an interval of 12 h between the morning and the evening milking. The individual production of milk was established at the outset of the trial, subsequently at intervals of 14 days and then at the end of the trial (nine controls).

The group feeding of the animals was *ad libitum* according to the unifeed technique. The foodstuffs were weighed each day, as were the residues from the previous day. The presence of these residues was always found to be very limited. The foodstuffs used were: second cut alfalfa hay, maize silage and two different concentrates specifically formulated. The latter were composed of the same feedingstuffs in different ratios, that is: soyabean meal (44%), maize meal (20%), barley meal (16%), wheat meal (5%), wheat middlings (4%), sunflower meal (3%), molasses (3%), and vitamin-mineral supplement (5%) for concentrate 1, and sunflower meal (26%), wheat middlings (26%), maize meal (12%), soyabean meal (9%), wheat meal (8%), barley meal (8%), dried alfalfa meal (3%), molasses (3%), and vitamin-mineral supplement (5%) for concentrate 2. The two diets administered had the same forage/concentrate ratio (53/47) but in their formulation the percentages of the two forages differed. Diet 1: alfalfa hay = 10%, maize silage = 43%, concentrate 1 = 47% (6.63 MJ/kg DM of net energy; 179.5 g/kg DM of crude protein). Diet 2: alfalfa hay = 20%, maize silage = 33%, concentrate 2 = 47%, (5.99 MJ/kg DM of net energy; 155.4 g/kg DM of crude protein). The net energy of the feeds, expressed as MJ/kg DM, was determined from the chemical composition and the digestibility of the organic matter (INRA, 1988).

Chemical analysis

Samples of forages and concentrates as well as residues were collected and these underwent the following analytical determinations: dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash according to AOAC (1995); neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) according to the Goering and Van Soest method (1970). In addition, the non-structural carbohydrates (NSC) were calculated as reported by Van Soest et al. (1991). The individual milk samples, representative of the yield of the two milkings, underwent the following analytical determinations: fat, protein (Nx6.38), casein, pH and urea (ASPA, 1995). In addition, three parameters of milk coagulation were established: rennet clotting time (r), curd firming time (K_{20}), curd firmness (A_{30}) by means of the thromboelastograph Formagraph as reported by Zannoni and Annibaldi (1981). In order to estimate the quantity of mozzarella cheese produced daily, the equation proposed by Altiero et al. (1989) was used. The fluoro-opto-electronic method (Somacounter 300, Bentley Instruments, USA) was employed on monthly samples to determine the somatic cell counts.

Statistical analysis

For the intake of dry matter, net energy and chemical

Table 1. Dry matter (g/kg as fed), chemical composition (g/kg DM) and net energy (MJ/kg DM) of feedstuffs utilized in experimental diets[1]

	DM	CP	CF	EE	NSC	Ash	NDF	ADF	ADL	NE_L
Feedstuffs:										
Alfalfa hay	884.6	149.3	396.6	12.0	255.4	89.8	493.5	455.3	104.0	4.69
Maize silage	298.4	81.5	269.9	25.5	263.1	62.0	567.9	347.1	46.1	5.69
Concentrate 1	901.8	275.7	72.5	26.9	437.8	86.1	173.5	89.1	11.4	7.90
Concentrate 2	905.3	209.9	120.3	29.6	375.5	90.1	294.9	147.2	47.5	6.76
Diets[2]:										
Group 1	640.6	179.5	189.8	24.8	344.5	76.1	375.1	236.7	35.6	6.63
Group 2	700.9	155.4	224.9	24.7	314.4	80.8	424.7	274.8	57.6	5.99

[1] DM = Dry matter; CP = Crude protein; CF = Crude fibre; EE = Ether extract; NSC = Non-structural carbohydrates; NDF = Neutral-detergent fibre; ADF = Acid-detergent fibre; ADL = Acid-detergent lignin; NE_L = Net energy.
[2] Composition on DM basis for Diet 1 (F:C 53:47): alfalfa hay = 10%, maize silage = 43%, concentrate 1 = 47%.
Composition on DM basis for Diet 2 (F:C 53:47): alfalfa hay = 20%, maize silage = 33%, concentrate 2 = 47%.

parameters, data from daily means of group were used whereas for live weight and milk quantitative and qualitative parameters, data from individual animals were utilized.

The differences between groups and between sub-periods were tested utilizing the GLM/SAS procedure (SAS, 1993) by a bifactorial model with interaction:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ijk}$$

where μ = general mean; α = group (i = 1, 2); β = sub-period (j = 1, 2); $(\alpha\beta)_{ij}$ = interaction group×sub-period; ε_{ijk} = error of model.

RESULTS AND DISCUSSION

Diet composition

Table 1 records the chemical composition, the net energy of the foodstuffs and the two diets utilized. The energy/protein content of Diet 1 (6.63 MJ/kg DM; 179.5 g/kg DM) does not appear in the tables of indicative requirements (Zicarelli, 2000; Bartocci et al., 2002; Scientific Committee of the Consortium for the Protection of Campania Buffalo Mozzarella Cheese, 2002). Diet 2, based on knowledge acquired during a previous study (Bartocci et al., 2006), has an energy/protein ratio (5.99 MJ/kg DM; 155.4 g/kg DM) deemed adequate. Considering the low level of structural carbohydrates supplied in Diet 1, the animals never had any metabolic or physiological problems.

Intake capacity

Table 2 presents the average daily intake of DM, NE_L, CP, structural and non-structural carbohydrates. Taking into consideration the entire period of the trial (33-146 DIM), Diet 1 did not modify the ingestion of dry matter which was

Table 2. Daily intake of dry matter, net energy, crude protein, structural and non-structural carbohydrates for entire period and interaction group×sub-period

		Entire period	Sub-periods		Rmse
			1st	2nd	
DMI (kg/d)	Group 1	17.23	16.61^B	17.85^A	0.82
	Group 2	17.29	16.59^B	17.97^A	
DMI/LW (%)	Group 1	$^B2.50$	$^B2.45^B$	$^B2.54^A$	0.12
	Group 2	$^A2.58$	$^A2.51^B$	$^A2.65^A$	
NE intake (MJ/d)	Group 1	$^A114.01$	$^A109.96^B$	$^A118.07^A$	5.33
	Group 2	$^B103.35$	$^B99.22^B$	$^B107.40^A$	
CP intake (kg/d)	Group 1	$^A3.09$	$^A2.98^B$	$^A3.20^A$	0.14
	Group 2	$^B2.69$	$^B2.58^B$	$^B2.79^A$	
NDF intake (kg/d)	Group 1	$^B6.46$	$^B6.23^B$	$^B6.70^A$	0.33
	Group 2	$^A7.34$	$^A7.05^B$	$^A7.63^A$	
NSC intake (kg/d)	Group 1	$^A5.94$	$^A5.72^B$	$^A6.15^A$	0.27
	Group 2	$^B5.44$	$^B5.22^B$	$^B5.64^A$	

DIM = Days in milk; DMI = Dry matter intake; LW = Live weight.
Means in the same row followed by different superscripts are significantly different (A, B: p<0.01).
Means in the same column preceded by different superscripts are significantly different (A, B: p<0.01).

similar for the two groups (17.23 and 17.29 kg/d), and these results concur with what was found by Bartocci et al. (2006); this research was undertaken under the same climatic conditions (spring ~ early summer) as the present trial utilizing pluriparous buffaloes with an average daily production for the previous year similar to that of the buffaloes used in the present study. The hypothesis can therefore be made that voluntary ingestion is not modified by an increase in the energy/protein level of the diet. According to Di Lella (1998), in the buffalo species, under the same physiological conditions, ingestion levels are almost always inferior to those detected for bovines. This result could be determined by the rate of transit of the foodstuffs with a slower ruminal transit (Bartocci et al., 1997) in the buffalo compared to the bovine. The difference between the ratio of ingestion of dry matter and the live weight (DMI/LW) of the two groups was significant (2.50 vs. 2.58%, p<0.01). These results concur with what was found in three experimental trials on lactating buffalo by Verna et al. (1994) where the ratio of dry matter/live weight varied from 2.2 to 2.6% depending on the type of forage utilized. Ranijhan (1992) also obtained the same variations while Bartocci et al. (2006) recorded a value of 2.43% for this parameter. On the contrary, Sarrubbi et al. (2000) and Infascelli et al. (2003) obtained an ingestion equal to 3.2% and 3.1% of live weight; those results were derived from the utilization of animals with a high productive capacity, not representative of the Italian buffalo population. The average daily intake of NE_L, CP and NSC was significantly (p<0.01) higher for Group 1, while adversely the intake of NDF was higher for Group 2 (p<0.01).

When considering the groups within the sub-period, there was no significant difference in the intake of dry matter; on the contrary, this parameter increased significantly (p<0.01), as did the DMI/LW ratio, passing from the first to the second sub-period for both groups. The peak intake was recorded at 115 and 123 days of lactation with 18.38 and 18.50 kg/d for the first and the second group, respectively, thus confirming the findings of Bartocci et al. (2006) of peak intake (17.40 kg/d) at 120 days. The average daily intake of NE_L, CP and NSC was significantly higher (p<0.01) in both the sub-periods for Group 1. On the contrary, NDF intake was always significantly (p<0.01) higher for Group 2. Passing from one sub-period to another there was a significant increase (p<0.01) in all the above-listed parameters for both groups.

Milk yield and milk composition

Table 3 presents the live weight, average daily gain, body condition score, milk yield and quality. Taking into consideration the entire period, no significant difference was noted between the average weights (689.7 and 669.4 kg), and the body condition scores (6.49 and 6.42) of the two groups; likewise the average daily gain (416 and 283 g/d), that determined an average increase of body weight of 47.42 and 32.26 kg, did not display any significant difference. Group 1, which received the diet with the higher energy/protein concentration, produced a greater quantity of milk (11.89 vs. 10.90 kg/d, p<0.10) of better quality both for its higher fat content (82.32 vs. 77.29 g/kg, p<0.10) and its protein content (47.36 and 46.38 g/kg) but with a greater fattening capacity of the buffaloes of 15.16 kg. Therefore Diet 1, with a higher energy input, determined not only a higher protein level, as for dairy cows, but there was also a tendency to higher level of fat. The increase of lipids has also been confirmed by other authors (Usmani and Inskeep, 1989; Verna et al., 1994; Bartocci et al., 2002). Bartocci et al. (2006), with a diet similar to that of Diet 1 for NE_L (6.69 MJ/kg DM) but with lower CP content (158.3 g/kg DM), achieved for the entire period of the trial (23-137 DIM) a milk yield similar to that of the group fed Diet 1 (11.66 kg/d) but of inferior quality with regard to fat content (73.20 g/kg), protein content (43.24 g/kg) and a lower average daily gain (307 g/d). Thus the greater quantity of protein of Diet 1 does not result in higher production but exclusively in better milk quality and an increased fattening of the animals. Comparing Diet 2 with that of previous work (Bartocci et al., 2006), there is a similar energy level (5.99 and 6.04 MJ/kg DM), but a different protein content (155.4 and 144.4 g/kg DM). This gives rise to a variation in the milk production of 10.90 and 9.42 kg/d, but a similar fat content (77.29 and 80.08 g/kg) and protein content (46.38 and 46.41 g/kg). Therefore, the difference in the protein level of the diet probably promoted galactopoiesis, increasing the quantity (+1.48 kg/d) but leaving the milk quality practically unchanged. This result reveals what was asserted by Campanile (1998) with regard to the onset of lactation and further conjectured by Bartocci et al. (2006) that, with a diet of 6.04 MJ/kg DM, the protein concentration must not be inferior to 150 g/kg DM. The casein content was similar for the two groups (41.36 and 40.37 g/kg) but higher than that obtained by Tripaldi (1994) and by Bartocci et al. (2006). This content was on average 87.16% of the protein content, while the previous authors noted 82.00 and 81.50%. The daily production of fat (0.97 vs. 0.83 kg/d, p<0.01), protein (0.56 vs. 0.49 kg/d, p<0.01) and casein (0.49 vs. 0.44 kg/d, p<0.05) was always statistically higher in favour of Group 1. With regard to milk urea, the highest value (39.47 mg/100 ml) was linked with the diet with the highest protein content, but the difference with Group 2 was not significant (38.12 mg/100 ml). Campanile et al. (1998), using a diet containing 120.0 g/kg of crude protein, noted a milk urea value equal to 35.00 mg/100 ml while Roy et al. (2005) obtained 44.82 and 42.53 mg/100 ml with diets containing 146.1 and 140.2

Table 3. Live weight, average daily gain, body condition score, milk yield and quality for entire period and interaction group x sub-period

		Entire period	Sub-periods		Rmse
			1st	2nd	
Live weight (kg)	Group 1	689.7	677.9	701.5	56.60
	Group 2	669.4	661.4	677.4	
Aver. daily gain (g)	Group 1	416	252^{b}	580^{a}	280.93
	Group 2	283	160	406	
BCS	Group 1	6.49	6.30	6.68	0.49
	Group 2	6.42	6.26	6.57	
Milk yield (kg/d)	Group 1	$^{\alpha}11.89$	$^{a}13.08^{A}$	10.71^{B}	1.55
	Group 2	$^{\beta}10.90$	$^{b}11.56$	10.24	
Fat (g/kg)	Group 1	$^{\alpha}82.32$	76.58^{B}	88.08^{A}	7.66
	Group 2	$^{\beta}77.29$	71.80^{b}	82.78^{a}	
Protein (g/kg)	Group 1	47.36	46.14^{b}	48.59^{a}	2.30
	Group 2	46.38	45.52	47.23	
Casein (g/kg)	Group 1	41.36	39.94^{b}	42.78^{a}	2.34
	Group 2	40.37	39.06^{b}	41.68^{a}	
Milk fat (kg/d)	Group 1	$^{A}0.97$	$^{A}1.00$	0.94	0.12
	Group 2	$^{B}0.83$	$^{B}0.83$	0.84	
Milk protein (kg/d)	Group 1	$^{A}0.56$	$^{a}0.60^{A}$	$^{\alpha}0.52^{B}$	0.06
	Group 2	$^{B}0.49$	$^{b}0.52^{\alpha}$	$^{\beta}0.46^{\beta}$	
Milk casein (kg/d)	Group 1	$^{a}0.49$	$^{a}0.52^{a}$	0.46^{b}	0.07
	Group 2	$^{b}0.44$	$^{b}0.45$	0.43	
Milk urea (mg/100 ml)	Group 1	39.47	42.21^{α}	36.73^{β}	5.98
	Group 2	38.12	39.73	36.51	

BCS = Body condition score.
Means in the same row followed by different superscripts are significantly different (α, β: p<0.10; a, b: p<0.05; A, B: p<0.01).
Means in the same column preceded by different superscripts are significantly different (α, β: p<0.10; a, b: p<0.05; A, B: p<0.01).

g/kg of crude protein. Bartocci et al. (2006), using diets containing 158.3 and 144.4 g/kg of crude protein, achieved similar milk urea values (39.09 and 39.05 mg/100 ml).

Within the two sub-periods, no significant difference emerged between the average weight, the average daily gain and the body condition score of the two groups. When considering the differences in the three above-listed parameters between the sub-periods, the only noteworthy finding to emerge from Diet 1, was that of average daily gain (252 vs. 580 g, p<0.05). Milk production, within the two sub-periods, was significantly different only for the first period in favour of Group 1 (13.08 vs. 11.56 kg/d, p<0.05). However, the positive effect of Diet 1 was neutralized (10.71 and 10.24 kg/d) in the second part of the trial. Consequently Diet 1, considering live weight, average daily gain and milk production, appears more appropriate only in the first ninety days of lactation. Figure 1 records the milk production of the two groups resulting from each control. The effect of Diet 1 was immediate with a peak of 14.00 kg/d after 47 DIM and with a successive production of approximately 13 kg/d, lasting up to 90 DIM. The same trend of milk production for Group 1, but at a lower level, was obtained by Bartocci et al. (2006) using 6.69 MJ/kg DM and 158.3 g/kg DM of crude protein, with a peak in production (13.13 kg/d) at 58 DIM. With Diet 2, there was a downturn (from 12.33 to 11.13 kg/d) with respect to the

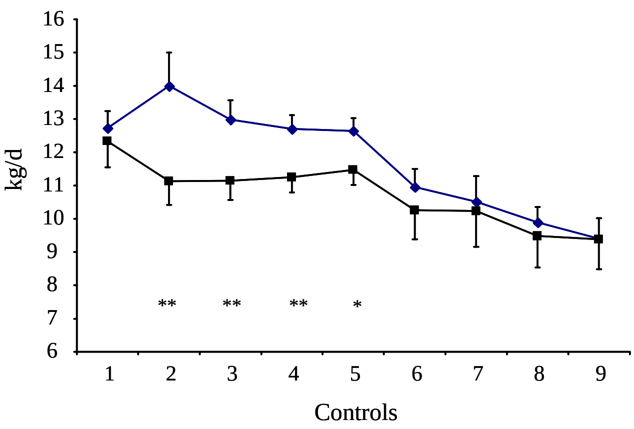

Figure 1. Milk production trends, kg/d (group 1 = ◆; group 2 = ■). * p<0.10; ** p<0.05.

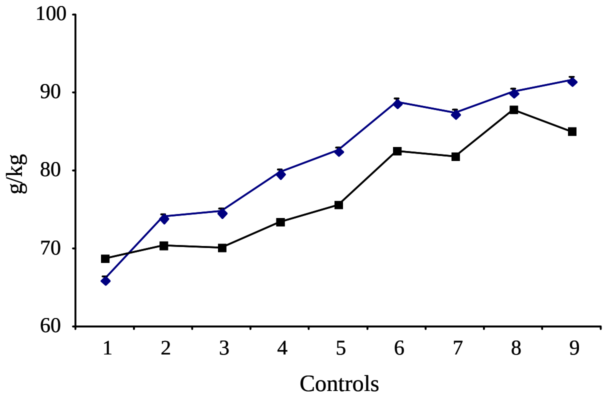

Figure 2. Trends in milk fat content, g/kg (group 1 = ◆;group 2 = ■).

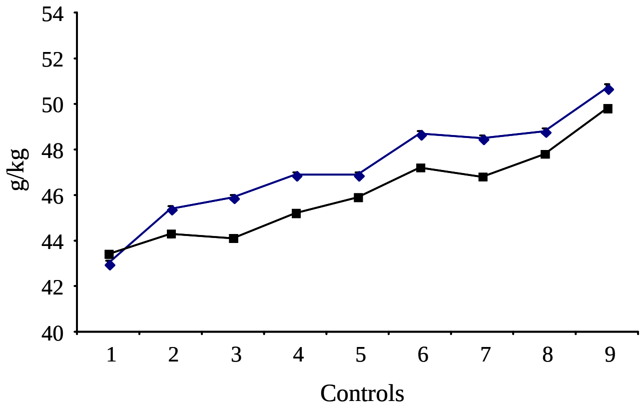

Figure 3. Trends in milk protein content, g/kg (group 1 = ◆; group 2 = ■).

initial data and production was maintained almost steady at this level up to 90 DIM; therefore, in order to achieve an increase in production during this phase of lactation, it is essential to utilize diets with a high energy/protein concentration. The difference in milk production between the two groups at each control in the first sub-period (33-90 DIM), turned out to be statistically significant in favour of Group 1; in the second sub-period, this difference was not significant, and progressively decreased until it was erased at the end of the trial. Therefore Diet 2, taking into consideration milk production, live weight and average daily gain, proved to be the most suitable for buffalo feeding from day 91 to 146 of lactation.

Within the two sub-periods (Table 3) there was no significant difference in the content of fat, protein or casein in the milk of the two groups, which was always higher in favour of Group 1. Comparing the first with the second sub-period, the content both in fat as well as in casein, increased significantly for both groups (fat: 76.58 vs. 88.08 g/kg, $p<0.01$, casein: 39.94 vs. 42.78 g/kg, $p<0.05$ for Group 1; fat: 71.80 vs. 82.72 g/kg, $p<0.05$, casein: 39.06 vs. 41.68 g/kg, $p<0.05$ for Group 2) while the protein content increased significantly only in Group 1 (46.14 vs. 48.59 g/kg, $p<0.05$). Considering the fat content (Figure 2) and protein content (Figure 3), control by control in both cases, these were always higher, although not significantly, in favour of Group 1. Therefore, the effect of the diet with the higher energy-protein concentration not only significantly increased the quantity of milk produced in the first part of the trial but also improved the quality for the entire duration of the research. Taking into consideration the average daily amount of fat, protein and casein within the two sub-periods, there was always a significant difference for all three parameters in the first sub-period, in favour of Group 1; in the second sub-period this applied only to the protein. In the two sub-periods there was no significant difference between the two groups with regard to the concentration of urea in the milk. With the passage from the first to the second sub-period, there was a decrease in this parameter which was significant only for Group 1 (42.21 vs. 36.73 mg/100 ml, $p<0.10$). This reduction was probably due to the significant increase of casein content in both groups.

Coagulation parameters and estimated yield of Mozzarella cheese

Table 4 lists the acidity, the coagulation parameters and the estimated yield of mozzarella cheese for both milks produced. Taking into account the entire period of the trial, the milk pH, 6.70 and 6.80 respectively for Groups 1 and 2, was significantly different ($p<0.01$). The pH level was inversely related to the energy level of the diet, as observed by Tripaldi (1994), and also to the casein content (41.40 and 40.50 g/kg), as established by Alais (1984) and by Tripaldi et al. (1997). Bearing in mind the three coagulation parameters of milk: rennet clotting time (r), curd firming time (K_{20}) and curd firmness (A_{30}), no significant difference was found. Nonetheless, the values of r (15.98 and 16.42 min) and K_{20} (1.66 and 1.75 min) were lower for the milk produced by Group 1. On the contrary, A_{30} (54.45 and 52.73 mm) was higher. A positive link between curd firmness and casein content was observed as already highlighted in buffalo milk by Tripaldi et al. (1997) and by Bartocci et al. (2006). From these findings it can be deduced that milk produced from the buffaloes fed with Diet 1 was not only of better quality, but also had a better aptitude for coagulation as determined by a low value of r, K_{20} and by a high value of A_{30}. The estimated mozzarella production was significantly higher in favour of Group 1 (3.05 vs. 2.59 kg/d, $p<0.01$); an intermediate value (2.71 kg/d) was obtained over the same period of lactation by Bartocci et al. (2006) with a diet composed of 6.69 MJ/kg DM of net energy and 158.3 g/kg DM of crude protein. No significant difference was found in the number of somatic cells: 141.22 and 152.74 n×1,000/ml respectively for the milk produced by

Table 4. pH, coagulating parameters and estimated yield of mozzarella cheese for entire period and interaction group×sub-period

		Entire period	Sub-periods		Rmse
			1st	2nd	
pH	Group 1	$^{B}6.70$	6.83^{A}	$^{B}6.57^{B}$	0.08
	Group 2	$^{A}6.80$	6.89^{A}	$^{A}6.71^{B}$	
r (min)	Group 1	15.98	18.44^{A}	13.54^{B}	2.66
	Group 2	16.42	19.09^{A}	13.76^{B}	
K_{2O} (min)	Group 1	1.66	1.77	1.54	0.42
	Group 2	1.75	1.82	1.67	
A_{30} (mm)	Group 1	54.45	48.47^{B}	60.42^{A}	5.67
	Group 2	52.73	47.25^{B}	58.21^{A}	
Estimated mozzarella cheese (kg/d)	Group 1	$^{A}3.05$	$^{A}3.22^{a}$	$^{a}2.87^{b}$	0.29
	Group 2	$^{B}2.59$	$^{B}2.70$	$^{b}2.48$	
Somatic cells (n×1,000/ml)	Group 1	141.22	126.81	155.64	62.14
	Group 2	152.74	127.21	178.28	

r = Rennet clotting time; K_{20} = Curd firming time; A_{30} = Curd firmness.
Means in the same row followed by different superscripts are significantly different (a, b: p<0.05; A, B: p<0.01).
Means in the same column preceded by different superscripts are significantly different (a, b: p<0.05; A, B: p<0.01).

Group 1 and 2.

In the two sub-periods, the pH of the milk was constantly higher in favour of Group 2, but with a significant difference only in the second sub-period (6.57 vs. 6.71, p<0.01). Passing from the first to the second sub-period, for both milks a significant reduction (p<0.01) was found in both pH (6.83 vs. 6.57; 6.89 vs. 6.71) and the values of r (18.44 vs. 13.54 min; 19.09 vs. 13.76 min) but no significant reduction was observed for K_{20}. On the contrary, a significant increase (p<0.01) of A_{30} (48.47 vs. 60.42 mm; 47.25 vs. 58.21 mm) was detected. Therefore both milks had a better aptitude for coagulation in the second part of the trial (91-146 DIM) and of the two, that of Group 1 always had the best capacity for coagulation. Estimated mozzarella yield was, in both sub-periods, significantly higher for Group 1.

CONCLUSIONS

The higher energy-protein level did not affect the intake of dry matter which remained fairly constant. This diet could be used exclusively during the first ninety days of lactation, with a positive effect on milk production and without any negative symptoms of a metabolic or physiological nature. The diet with the lower energy-protein content could be used from the 90^{th} to at least the 150^{th} day of lactation.

The milk produced by both groups was of a good quality, but the best was from the buffaloes fed with the diet with the highest energy and protein content, which displayed a greater aptitude for coagulation.

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REFERENCES

Alais, C. 1984. Science du lait. Principes des techniques laitières. SEPAIC, Paris, France.

Altiero, V., L. Moio and F. Addeo. 1989. Estimate of mozzarella yield based on the fat and protein content of buffalo milk. Sci. Tecn. Latt.-Cas. 40:425-433.

AOAC. 1995. Official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington, DC.

ASPA. 1995. Methods of milk analysis of the main zootechnical species. Università degli Studi di Perugia, Italy.

Bartocci, S., A. Amici, M. Verna, S. Terramoccia and F. Martillotti. 1997. Solid and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livest. Prod. Sci. 52:201-208.

Bartocci, S. and S. Terramoccia. 2006. Effect of NDF/undegradable crude protein ratio on *in vivo* digestibility, particle passage rate in riverine buffaloes compared with sheep. Livest. Sci. 104:38-45.

Bartocci, S., S. Terramoccia and C. Tripaldi. 2006. The utilisation of a high level energy/protein diet for lactating Mediterranean buffalo: Intake capacity and effects on quanti-qualitative milk parameters. Livest. Sci. 99:211-219.

Bartocci, S., C. Tripaldi and S. Terramoccia. 2002. Characteristics of feedstuffs and diets, and the quanti-qualitative milk parameters of Mediterranean buffaloes bred in Italy using the intensive system. An estimate of the nutritional requirements of buffalo herds lactating or dry. Livest. Prod. Sci. 77:45-58.

Bittante, G., M. Ramanzin, L. Bailoni, A. Simonetto and S. Bartocci. 1994. Confronto fra alcuni parametri ruminali di bufalini, bovini e ovini alimentati con diete diverse. Agricoltura Ricerca 153:135-142.

Campanile, G. 1998. Relationship between nutrition and reproduction in buffalo. *Bubalus Bubalis* 1:217-235(Suppl).

Campanile, G., C. De Filippo, R. Di Palo, W. Taccone and L. Zicarelli. 1998. Influence of dietary protein on urea levels in blood and milk of buffalo cows. Livest. Prod. Sci. 55:135-143.

de Stefano, F. 1999. Ottime potenzialità per l'allevamento bufalino in Italia. L'informatore agrario 51:59-62.

Di Lella, T. 1998. Buffalo nutrition. Bubalus Bubalis 1:207-216 (Suppl).

Goering, H. K. and P. J. Van Soest. 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). ARS USDA Agriculture Hand Book N° 379, Washington, DC.

Infascelli, F. 2003. New acquisitions on the nutrition and feeding of buffalo. Atti 2° Congresso nazionale sull'allevamento del bufalo. 28-30 Agosto 2003, Monterotondo-Roma, Italy, pp. 1-18.

INRA. 1988. Alimentation des bovins, ovins et caprins. INRA Publ., Paris, France.

Kennedy, P. M., A. N. Boniface, Z. J. Liang, D. Muller and R. M. Murray. 1992. Intake and digestion in swamp buffaloes and cattle. 2. The comparative response to urea supplements in animals fed tropical grasses. J. Agric. Sci. Camb. 119:243-254.

Nisa, M., A. Javaid, M. Aasif Shahzad and M. Sarwar. 2008. Influence of varying ruminally degradable to indegradable protein ratio on nutrient intake, milk yield, nitrogen balance, conception rate and days open in early lactating *Nili-Ravi* buffaloes (*Bubalus bubalis*). Asian-Aust. J. Anim. Sci. 21:1303-1311.

Ranjhan, S. K. 1992. Nutrition of river buffaloes in Southern Asia. In: Buffalo production (Ed. N. M. Thulloh and J. H. G. Holmes). Elsevier, Amsterdam, pp. 117-134.

Roy, B., R. K. Mehla and S. K. Sirohi. 2005. Effect of dietary feeding regimens on urea and protein concentration of milk in Murrah buffaloes. Asian-Aust. J. Anim. Sci. 18:973-979.

Sarubbi, F., T. Di Lella, G. Galiero, F. Infascelli, F. Bovera and G. Durante. 2000. Feeding diets varying in crude protein concentration and ruminal degradability to lactating Mediterranean buffalo cows. Zoot. Nutr. Anim. 26:181-188.

SAS Statistical Analysis System Institute. 1993. SAS User's guide, Statistics, Sas Institute Inc., Cary, NC, USA.

Scientific Committee of the consortium for the protection of Campania buffalo mozzarella cheese (Ed. S. Bartocci, G. Campanile, F. Consalvo, E. Correale, A. Di Francia, V. Proto, S. Terramoccia and L. Zicarelli). 2002. "Pattern of regulation for sanitary and feeding management of buffalo herds for the production of buffalo mozzarella cheese". Ed. Consorzio Tutela Mozzarella di Bufala Campana, S. Nicola la Strada (CE), Italy.

Terramoccia, S., S. Bartocci, A. Amici and F. Martillotti. 2000. Protein and protein-free dry matter rumen degradability in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livest. Prod. Sci. 65:185-195.

Tripaldi, C. 1994. Effetto di alcune diete sulle caratteristiche qualitative del latte di bufala. Agricoltura Ricerca 153:79-86.

Tripaldi, C., G. Catillo, F. Martillotti and M. Angelucci. 1997. Influence of some characteristics of diet on milk quality of water buffalo. Buffalo J. 13:1-13.

Usmani, R. H. and E. K. Inskeep. 1989. Effect of prepartum feeding on milk yield and calf growth rate in limited-suckled and nonsuckled buffaloes. J. Dairy Sci. 72:2087-2094.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Verna, M., S. Bartocci, A. Amici and M. Agostani. 1994. Effect of different diets on productive performance of lactating buffaloes. Agricoltura Ricerca 153:73-78.

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann and L. E. Walters. 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603-612.

Zannoni, M. and S. Annibaldi. 1981. Standardization of the renneting ability of milk by Formagraph. Sci. Tecn. Latt.-Cas. 32:79-94.

Zicarelli, L. 2000. Nutrition in dairy buffaloes. Ed. Università degli studi di Napoli Federico II. Facoltà di Medicina Veterinaria.