Effect of Dietary Energy and Protein Contents on Buffalo Milk Yield and Quality during Advanced Lactation Period

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ABSTRACT : Among Italian buffalo farmers, it is widely held that administering diets with high energy and protein concentrations is an effective way to increase milk production. In order to assess the validity of this opinion, we verified milk yield and physico-chemical characteristics from buffaloes that, from the 5th month of lactation, were fed two total mixed rations (TMRs) which, given the same intake, should have led to satisfaction of protein requirements though with a slight energy deficit (diet A) or excessive amounts of energy and protein (diet B). Estimate of the energy and protein value of the diets and that of the corresponding requirements was carried out both by using two software programs derived from the Cornell Net Carbohydrate and Protein System (1992), and with the method set up by INRA researchers (1988). The results obtained show that the two diets administered did not result in significant changes to the quantity of milk produced. However, with Diet B the protein concentration in the milk was significantly ($p<0.01$) higher, although this was partly offset by the higher concentration ($p<0.05$) of non-protein nitrogen (NNP). The Group B buffaloes also showed significantly higher blood urea levels ($p<0.01$), with concentrations exceeding those considered physiological for lactating buffaloes. Finally, while administering Diet A the Body Condition Score (BCS) was close to 6.5 (Wagner et al., 1988), whereas in buffaloes which used Diet B it sometimes increased by over 0.5 points. As regards which of the two methods compared is more suitable for expressing dietary energy and protein value and corresponding requirements, we feel that due to the high variability in the Italian Mediterranean buffalo's milk production aptitude, it would be premature to express a judgement on methods which rest on a common scientific base and do not differ substantially. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 675-681)***

Key Words : Italian Mediterranean Buffalo, Milk Production, Dietary Energy and Protein Level

INTRODUCTION

In Italy the buffalo sector is in continuous expansion: buffalo milk is in great demand and reaches high prices since it is used for the production of "Buffalo mozzarella from Campania", a stretched curd cheese to which the European Union has awarded the POD brand (Protected Origin Denomination). As this animal species has only recently become the focus of livestock breeding in Italy, its milk producing aptitude still varies greatly. This induces buffalo farmers to capitalize on the healthy profit margins from milk sales and seek to maximize production, supplying diets that, in each lactation phase, ensure energy and protein intake which exceeds corresponding requirements. It therefore seemed useful to study, in buffaloes that were in their 5th month of lactation when the groups were formed, the effects of feeding two different diets: one which would give rise to a slight energy deficit but a small protein surplus; the other formulated to provide a surplus intake of both energy and protein.

A further aim of the study was to compare the method set up by INRA researchers (1988), which is still the most commonly used in Italy to express dietary energy and protein value for ruminants and corresponding requirements, with the Cornell Net Carbohydrate and Protein System (Russel et al., 1992; Sniffen et al., 1992; Fox et al., 1992) which, thanks to the diffusion of various computer programs, is being increasingly used in cattle dairy farming.

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MATERIALS AND METHODS

Animals

The study was carried out on a private farm situated close to Paestum (Salerno), which is the top area in Italy in terms of average yield/lactation (about 3,400 kg of milk). A total of 24 buffalo cows were used in their 5th month of lactation, weighing 640±26 kg, and with a Body Condition Score (BCS) close to 6.5, assessed with the 1-9 scale proposed by Wagner et al. (1988). The animals were subdivided into two groups as homogeneous as possible in calving order (between 2nd and 4th), in daily milk production (9.54±2.41 kg vs 9.85±2.62 kg), as well as percentages of fat (8.00±1.05 vs 7.82%±1.31) and protein (4.47±0.34 vs 4.11%±0.34), in Groups A and B, respectively.

Converted into FPCM (Fat and Protein Corrected Milk) using Di Palo's equation (1992):

$$Y=1+0.01155\ [(X-40)+(Z-31)]$$

where:
Y is the quantity (kg) of FPCM equivalent to 1 kg of milk produced;
X and Z are the grams of fat and protein contained in 1 kg

of milk produced;

the yields were: 15.52±2.82 kg for Group A and 15.31± 3.10 kg for B.

For the application of the Cornell Net Carbohydrate and Protein System (CNCPS) we used two software programs: CPM-Dairy (1998), which had already been used in formulating diets for lactating buffaloes (Bovera et al., 2000), and CNCPS-version 4.0.29 (2000).

In estimating energy and protein requirements, the amounts needed for maintenance were identified with the requirements that each of the programs used and the INRA method attribute to dairy cows with identical weight, BCS, lactation phase and yield level to those of the buffalo cows in question.

Instead, to calculate production requirements we followed the guidelines of Di Lella (1998), according to which the Italian Mediterranean buffalo, in order to produce 1 kg of FPCM, needs 3.56 MJ of milk net energy instead of 3.13, as estimated for dairy cows. Under the same guidelines, the efficiency with which the buffalo converts metabolizable proteins into milk proteins (N×6.38) is 50% rather than 70%, as obtained by the CNCPS (Fox et al., 1992); also the conversion of PDIs into milk proteins is 50% efficient in buffalo compared with 64% in cattle (INRA, 1988). Such lower efficiencies are thought to be due to the fact that in the Italian Mediterranean buffalo the milk production aptitude is still highly variable.

Thus, to take account of such reduced efficiencies, requirements for Group A buffaloes were assumed to correspond to those of dairy cows with average daily production of 17.63 kg of milk, at 4.00% fat and 3.38% protein. Calculation relative to group A. FPCM production: 15.52 kg; estimated requirement for buffaloes: 3.56 MJ/kg FPCM; 15.52×3.56=55.2 MJ; requirement for cows: 3.13 MJ/kg FPCM; 55.2/3.13=17.63 kg milk. Protein 44.7 g/kg milk; total protein 9.54×44.7=426.4 g; estimated yield in buffaloes 50%: 426.4×0.5=852.8 g; estimated yield in cows 70%: 852.8×0.7=597.0 g; 597/17.63=33.8 g/kg milk. Applying the same criteria for the Group B buffaloes, requirements were identified with those of dairy cows producing an average 17.41 kg of milk daily, at 4.00% fat and 3.26% protein. When the INRA method was used to calculate requirements, we assumed that protein concentrations of 3.10% for Group A and 2.98% for Group B corresponded to the quantities listed above.

Diets

In formulating the two Total Mixed Rations (TMRs), each labeled with the same letter as that identifying the buffalo group to which it was administered, we ensured that the daily intake of about 15.5 kg DM/head, using the two software packages from the Cornell method, determined daily and in each animal an energy deficit close to 6.0 MJ of NE_l and a surplus (about 100 g) of metabolizable proteins (MP) with Diet A; by contrast, with B, we arranged for a slight surplus of NE_l but a substantial surplus of MP.

The components (% DM) of the two TMRs are given in table 1; their chemical composition, determined according to the Food Evaluation Commission of ASPA (1980), Martillotti et al. (1987) and with regard to carbohydrate and protein fractions, according to Sniffen et al. (1992), is listed in table 2.

The energy and protein contents of the dry matter of each TMR, estimated with the two compared methods, are given in table 3. They were calculated by inserting in each database the results of chemical determinations which we performed also on the individual ingredients used in formulating the compound feeds. In table 3 we also give the percentages of bacterial metabolizable protein (BMP), as well as those of degradable proteins (DIP) and soluble proteins (SP), referring to crude proteins. As regards the INRA method, for each TMR we indicate the quantities (g/kg DM) of protein digested in the small intestine supplied by rumen-undegraded dietary protein (PDIA), and available global proteins (microbial+dietary) depending on whether microbial development was limited by energy (PDIE) or by nitrogen (PDIN).

Finally, table 4 gives the requirements, estimated by taking account of the buffalo's lower efficiency in converting energy and protein, and the corresponding energy and protein levels permitted by average intake recorded in each group and estimated using CPM-Dairy, the CNCPS and the INRA.

Experimental procedures

After formation of the two groups and throughout the study period (15 d of adaptation and 42 in the study phase) the two TMRs were supplied within a single daily feed (15.5 kg di DM/head). Moreover, as the farm had a computerized feeding system, it was possible to record

Table 1. Diet components (% DM)

	Diet A	Diet B
Corn silage	39.7	40.3
Oat hay	22.0	22.3
Concentrate	38.3[1]	37.4[2]

[1] Concentrate diet A components (% DM): wheat middlings, 31.3; sunflower meal, 26.3; beet pulp, 21.2; soybean meal, 11.8; corn gluten meal, 6.1; calcium carbonate, 1.1; mix bovine, 1.1; salt, 1.1.

[2] Concentrate diet B components (% DM): soybean meal, 27.7; corn, 21.5; extruded soybean, 16.0; beet pulp, 15.7; sunflower meal, 10.7; corn gluten meal, 5.1; calcium carbonate, 1.1; mix bovine, 1.1; salt, 1.1.

Table 2. Chemical and nutritional characteristics of dietary components (g/kg DM)

	Corn silage	Oat hay	Concentrate A	Concentrate B
Moisture, g/kg t.q.	696	109	104	107
Crude protein	71	66	245	291
Ether extract	30	17	31	59
Crude fiber	197	353	147	105
Ash	58	119	88	92
Total carbohydrates	740	798	636	658
NDF	410	618	362	241
ADF	230	481	186	144
ADL	18	61	29	22
Cellulose	199	380	146	120
NSC	440	206	304	346
			Carbohydrate fractions	
Fraction A	169	169	158	173
” B_1	271	37	146	173
” B_2	257	446	262	259
” C	43	146	70	53
			Protein fractions	
Fraction A	21	18	31	29
” B_1	12	3	19	20
” B_2	29	18	165	214
” B_3	3	19	5	2
” C	6	8	25	26

Table 3. Energy and protein value of dry matter of TMR according to the three methods used

	CPM		CNCPS		INRA	
	A	B	A	B	A	B
NE_l, MJ/kg DM	6.02	6.78	6.36	7.07	5.81	6.69
MP, g/kg DM	99.6	106.4	102.8	109.1	-	-
BMP, g/kg MP	69.6	64.5	67.6	61.1		
DIP, % CP	64.2	64.1	67.3	65.9	-	-
SP, % CP	28.2	25.9	30.0	27.8	-	-
PDIA, g/kg DM	-	-	-	-	32.5	60.5
PDIE, g/kg DM	-	-	-	-	88.9	110.0
PDIN, g/kg DM	-	-	-	-	90.1	106.8

MP=Metabolizable protein; BMP=bacterial metabolizable protein; DIP=protein into rumen degradable; SP=soluble protein; PDIA= protein digested in the small intestine supplied by rumen-undegraded dietary protein; PDIE and PDIN=PDIA plus PDI supplied by microbial proten from rumen fermented organic matter or rumen degraded protein.

individual production at each milking. By contrast, milk samples were taken from each buffalo at group formation (beginning of the adaptation phase) and at the beginning and end of the study period. All the samples were promptly transferred to the Department laboratories and used to determine the following: concentrations of fat, protein and lactose using a milk analyzer (Milko Scan 133 BN Foss Electric, Hillerod, Denmark) calibrated with appropriate buffalo milk standards; non-casein N (NNC) and non-protein N (NNP), using the Kjeldhal method according to ASPA guidelines (1995); casein (total N-NNC)×6.38 (ASPA, 1995). Each sample was also used to measure pH and titratable acidity (°SH, ml of NaOH/100 ml of milk) by means of titration with NaOH N/4 (ASPA, 1995). At the beginning and end of the study period, in the morning and before feeding, blood samples were also taken from the jugular vein to determine concentrations of urea, AST, ALT, (Reflotron automatic analyzer, using the relative Roche kits) and bilirubin (ECHO ISE-EDIF automatic analyzer, Firmware 2.37).

To convert into FPCM the milk production from the adaptation and study period, we used concentrations of fat and proteins corresponding to the average contents determined at the beginning and end of each of the above periods.

Table 4. Energy and protein requirements and corresponding contribution, estimated by three methods

	CPM		CNCPS		INRA	
	A	B	A	B	A	B
			Energy requirements - NE_l, MJ/d			
Maintenance	43.5	43.5	47.8	48.8	41.3	41.3
Production	54.8	54.0	55.3	54.1	56.9	54.1
Total	98.3	97.5	103.1	102.9	98.2	95.4
			Amounts permitted by rations - NE_l, MJ			
	92.2	102.3	97.3	106.8	89.3	101.0
			Protein requirements, g/d			
	MP		MP		PDI	
Maintenance	591	561	606	538	415	415
Production	853	811	876	812	853	809
Total	1,444	1,372	1,482	1,350	1,268	1,224
			Amounts permitted by rations, g			
	1,524	1,607	1,573	1,647	1,360	1,613

Statistical analysis

Individual daily production, real and converted to FPCM, and the results of analyses conducted on milk and blood samples, were elaborated statistically by using the GLM procedure of the SAS (2000), according to the following model:

$$Y_{ijk}=m+D_i+P_j+D\cdot P_{ij}+e_{ijk}$$

where:
Y_{ijk}=each observation;
m=general mean;
D_i=effect of diet (i = 1, 2);
P_j=according to cases, effect of the lactation period (j=1 adaptation, 2 study period); effect of milk sample (j=1, …, 3); effect of blood sample (j=1, 2);
$D\cdot P_{ij}$=interactions between diet and lactation period, or between diet and samples;
e_{ijk}=error.

RESULTS AND DISCUSSION

Daily monitoring of food residues, never exceeding 4.2 kg DM in Group A or 5.8 kg in B, allowed average DM intake to be calculated (15.3 kg in the first and 15.1 kg in the second group), consumption commonly found in buffaloes at the 5th-6th month of lactation, with production close to our findings.

Therefore, as indicated in table 4, with both programs derived from the Cornell system, Diet A resulted in a daily NE_l deficit close to 6 MJ/head, but also slight surpluses of metabolizable proteins (80-90 g). With Diet B, besides the considerable surpluses of metabolizable protein (about 230 g, according to the CPM; almost 300 g by the CNCPS), slight NE_l surpluses (5-4 MJ) were also recorded. Estimates of the energy and protein value of the two diets and the corresponding requirements using the INRA method supplied results which agreed with those given above, although deficits and surpluses showed somewhat different quantities.

Table 5 gives the percentage contents of fat and protein as determined in individual milk samples taken at the end of adaptation and at the end of the study period. Statistical elaboration showed that of the two main effects tested, only diet resulted in a significant difference ($p<0.01$) between protein contents, which were higher in Group B.

Analysis of daily milk production in the adaptation period and the experimental period supplied somewhat unexpected results (table 6), in relation to the different intakes permitted by the diets. Higher energy and protein intakes did not result in statistically significant yield increases: on the contrary, it was the buffaloes fed Diet A that showed higher milk production. However, if this is converted into FPCM on the basis of fat and protein contents relative to the adaptation and study periods, it was the Group B buffaloes which supplied the greater yields. As expected, as lactation progressed (period effect) significant reductions were recorded ($p<0.05$) in real and FPCM production. Finally, in no case was there a significant diet x period interaction.

Table 5. Individual milk fat and protein contents at the beginning (2nd sampling) and end (3rd sampling) of the experimental period(1)

	Effect of diet		Effect of sample		SEM DF=44
	A	B	2nd	3rd	
Fat, %	7.21	8.07	7.73	7.55	2.407
Crude protein, %	4.10^B	4.50^A	4.41	4.19	0.180

^{A,B} $p<0.01$.
(1) There were no significant interactions.

Table 6. Average individual actual yield and FPCM, according to diet and period[(1)]

	Effect of diet		Effect of period		SEM DF=44
	A	B	Adaptation	Trial	
			Real milk yield		
kg/d	8.57	8.24	8.98[a]	7.83[b]	2.73
			FPCM		
kg/d	12.87	13.52	14.21[a]	12.18[b]	8.27

[a,b] $p<0.05$.
FPCM=Fat and protein corrected milk.
[(1)] There were no significant interactions.

Table 7. Other chemical and physical characteristics of milk produced by the two groups of buffaloes in various phases of the trial

Diets	A		B	
	Mean±SD		Mean±SD	
			1st sampling-Group formation	
DM	17.81	2.24	17.84	1.60
Ash, %	0.98	0.17	1.08	0.14
Lactose, %	4.43	0.66	4.83	0.61
Casein, %	3.64	0.28	3.38	0.33
pH	6.64	0.08	6.69	0.12
Acidity, °SH	8.37	0.98	8.67	1.47
			2nd sampling-End of adaptation period	
DM	17.10	1.75	18.91	2.21
Ash, %	0.93	0.14	1.072	0.23
Lactose, %	4.81	0.58	4.90	0.74
Casein, %	3.34	0.28	3.83	0.32
pH	6.67	0.07	6.71	0.09
Acidity, °SH	8.75	0.92	9.46	1.25
			3rd sampling-End of trial period	
DM	17.16	1.35	17.87	1.55
Ash, %	0.97	0.05	0.95	0.06
Lactose, %	4.91	0.70	4.71	0.74
Casein, %	3.29	0.56	3.55	0.37
pH	6.57	0.12	6.54	0.10
Acidity, °SH	8.42	1.00	8.81	1.15

During the two-month study period, no substantial changes were noted in the BCS of buffaloes using diet A, while that of the Group B buffaloes increased, at times by over 0.5 points.

Table 7 presents the measurements of dry matter, ash, lactose, casein, pH and titratable acidity in the individual milk samples taken when the groups were formed and at the beginning and end of the experimental period. Statistical analysis showed no significant differences according to the main factors considered. However, the casein content which was higher in milk produced by Group A at the first sampling, was higher in both subsequent samplings in the milk of Group B. However, significant differences were recorded as regards the NNP content (table 8), which was higher ($p<0.05$) in the milk of buffaloes using the diet which permitted higher intakes of NE_l and proteins.

Finally, table 9 gives the results of measurements made at the beginning and end of the study period on blood samples so as to ascertain the concentrations of urea and of several enzymes, which may be used to monitor liver functionality of the animals in question. In lactating buffaloes blood urea concentrations between 5 and 7.5 mM are considered physiological (ASPA, 1999). While the values recorded in buffaloes fed with Diet A lie at the upper limits of this range, those from Group B appear well outside it, close to 9 mM. The high levels encountered in both groups appear in line with the energy and protein balances indicated in table 4 and with the fact that, due to the

Table 8. NPN concentrations in milk produced by the two buffalo groups in various phases of the trial[(1)]

Effect of diet		Effect of sampling[(2)]			SEM DF = 66
A	B	1th	2th	3th	
0.204[b]	0.219[a]	0.210[AB]	0.220[A]	0.205[B]	0.69[-3]

[A,B] p<0.01; [a,b] p<0.05.
[(1)] There were no significant interactions
[(2)] 1th: Group formation; 2th beginning experimental period; 3th end experimental period.

Table 9. Concentrations of urea, bilirubin and AST/ALT ratio in blood samples from the two groups of buffaloes at the beginning and end of the trial period[(1)]

	Effect of diet		Effect of sampling		SEM
	A	B	1th	2th	DF=44
Urea, mM	7.57[B]	8.93[A]	8.35	8.15	1.47
Bilirubin, µM	3.64	3.60	2.75[B]	4.49[A]	1.33
AST/ALT	2.09[a]	2.55[b]	2.37	2.27	0.365

[A,B] p<0.01; [a,b] p<0.05.
[(1)] There were no significant interactions.

progressive reduction in yields, the energy requirements were substantially satisfied also with Diet A. Indeed, in our opinion, the significantly higher blood urea levels recorded (p<0.01) in buffaloes fed Diet B, are to be ascribed to high protein intake resulting from the diets used.

The concentrations of bilirubin do not appear to be significantly affected by diet, although they show a significant increase (p<0.01) between the beginning and end of the study period. Given that the range of normal values in the lactating buffalo oscillates between 2.5 and 4.0 µM (ASPA, 1999), only at the end of the trial period were average concentrations above this range. Since, from our experience (unpublished data), in the second half of pregnancy buffalo cows frequently have bilirubin concentrations which are far in excess of 4 µM, we believe that the values encountered at the end of the trial should be attributed mainly to the fact that in this phase almost all the buffaloes were in their 3rd-4th month of pregnancy.

The AST/ALT ratio is considered by some (ASPA, 1999) to be of greater use, with respect to concentrations of each of the two enzymes, for evaluating whether there are conditions of suffering or liver damage. Despite showing significant modifications (p<0.05) as a function of diet, it never reached values which indicated altered liver functionality.

CONCLUSIONS

Our results show that in buffaloes in advanced lactation, surplus amounts of energy and protein do not bring about significant changes in the quantity of milk produced, compared with when the forage/concentrate ratio and dry matter intake are left almost unchanged, and a ration is supplied which can satisfy the animal's protein requirements but falls slightly short of the energy requirements. It was also noted that buffaloes enjoying ample energy and protein intake supplied milk with a significantly (p<0.01) higher percentage crude protein. This observation is of considerable importance in the case of buffalo milk, used exclusively for cheese-making in Italy. However, this is offset by the fact that also the NNP content was found to be significantly higher (p<0.05). Moreover, the animals that used the diet with a higher protein concentration also had significantly higher (p<0.01) blood urea concentrations, which were outside the normal range of physiological levels in lactating Italian Mediterranean buffaloes.

Finally, the high variability in the aptitude of buffaloes raised in Italy for milk production and the common scientific base of the two methods which we tested to express dietary energy and protein values and corresponding requirements, led us to believe that at the moment, there were no sound assessment to express a judgement on which methods is to prefer.

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