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SHORT COMMUNICATION

Prediction of milk, fat and protein yields in first lactation from serum ß-lactoglobulin concentrations during gestation in Italian Brown heifers

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ABSTRACT

The Authors report the results of a study carried out on 23 pregnant Italian Brown heifers, with the aim to determine the relationships between blood serum ß-lactoglobulin (ß-LG) concentrations during first gestation and subsequent milk production and quality in first lactation, in order to obtain an improved selection method for replacement heifers. At weeks 20, 26 and 32 of gestation, ß-LG concentrations (±SE) were 706±78, 753±66 and 772±63 ng/ml, respectively (P>0.05). High and significant (P≤0.05) correlation coefficients were observed only between ß-LG content at week 32 and total milk and protein yields in first lactation. Prediction equations of milk, fat and protein production in first lactation from $\log_{10}$ ß-LG content at week 32 of gestation, from parent average genetic indexes and from both were calculated by means of multiple regression analysis. When the contribution of both ß-LG content and predicted genetic indexes were considered, the regression equations gave generally a better estimate of the production parameters in first lactation (higher R^2, lower SE of estimate) than the above mentioned parameters alone. These results suggest that it is valuable to pre-estimate milk, fat and protein production in Italian Brown first lactating cows by means of the analysis of serum ß-LG content during gestation.

Key words: Dairy cattle, Italian Brown breed, ß-lactoglobulin, Milk production, Prediction.

RIASSUNTO

IL CONTENUTO DI ß-LATTOGLOBULINA SIERICA DURANTE LA GRAVIDANZA QUALE INDICATORE DELLA PRODUZIONE DI LATTE, GRASSO E PROTEINE IN PRIMA LATTAZIONE IN BOVINE DI RAZZA BRUNA ITALIANA

È stata condotta una prova su 23 manze di razza Bruna Italiana con lo scopo di determinare i rapporti fra il contenuto di ß-lattoglobulina (ß-LG) nel siero nel corso della gravidanza e la produzione quanti-qualitativa del latte in prima lattazione. Il contenuto medio di ß-LG a 20, 26 e 32 settimane di gravidanza è risultato pari a 706±78, 753±66 e 772±63 ng/ml (P>0,05). Il contenuto di ß-LG alla 32ª settimana di gestazione è risultato significativamente (P<0,05) correlato solo con la produzione totale di latte e di pro-

teine in prima lattazione. Mediante l'applicazione della regressione multipla, sono state calcolate le equazioni di previsione della produzione quanti-qualitativa di latte in prima lattazione a partire dal contenuto di ß-LG alla 32ª settimana di gestazione, dal valore dei diversi indici genetici di previsione e da entrambe le tipologie di caratteri. In quest'ultimo caso la bontà della previsione è risultata migliore (R^2 più elevati, minor errore standard della stima) rispetto all'uso degli stessi da soli. I risultati indicano la possibilità di migliorare la stima della futura produzione lattea nella manza, per mezzo dell'analisi del contenuto di ß-LG del siero ematico alla 32ª settimana di gravidanza.

Parole chiave: *Bovina da latte, Razza Bruna Italiana, ß-lattoglobulina, Produzione di latte, Previsione.*

Introduction

Replacement heifer management is very important in dairy herds. Milk produced in first lactation is sufficient to counterbalance the expenditure during the growing period, if the first parturition occurs at least at 24 months of age (Campiotti and Calamari, 2001). However, the future producing ability of a heifer is unknown and performance in first lactation is often different from what is expected on the basis of the pedigree index alone. Thus, the selection of replacement heifers could be improved by the use of physiological markers of milk production and quality (Schanbacher and Smith, 1975).

Beta-lactoglobulin (ß-LG) is the main fraction of milk whey proteins in most ruminant species (Eigel *et al.*, 1984). Its production during gestation exhibits a log-linear increase, that reflects the growth and development of the mammary gland (Mao *et al.*, 1991). Farrell and Thompson (1990) first suggested that both ß-LG and α-lactalbumin could be potential modulators of mammary cell activity. However it is not clear if the abovementioned whey proteins have a direct effect on mammary function. During gestation ß-LG (as other whey proteins) originating from the interstitial fluid can be found in blood serum, because the tight junctions between the secretory mammary cells are not completely held together until just prior to parturition (Mao and Bremel, 1995). So their content in blood serum during gestation could be considered as a physiological marker of mammary growth and development.

In a previous study (Sabbioni *et al.*, 2004) the relationship between serum ß-LG content during gestation and subsequent reproductive efficiency in primiparous sows was studied. The results indicated that serum ß-LG content during the final weeks of gestation could be used as an early indicator of reproductive efficiency, and that gilts with high contents produce heavier litters (+17%) and show higher milk production (+23%) during the first 5 d after farrowing. Thus they could be selected to improve herd productivity.

Mao and Bremel (1995) reported that blood serum ß-LG content at week 26 of gestation in pregnant Holstein heifers with high genetic merit could predict milk production during first lactation.

No information is actually available for other breeds with lower production levels but higher milk fat and protein content. The aim of the present research was to assess the possibility of using ß-LG content during gestation in Italian Brown heifers as a physiological marker of future milk production and quality in first lactation.

Material and methods

The trial was carried out on 23 Italian Brown heifers from 14 sires, homogeneous with reference to age and weight, reared in one herd in the Province of Parma. The heifers were inseminated at a mean age of 654±14 d. Blood samples were collected by needle puncture in the coccygeal vein at 1000 h at weeks 20, 26 and 32 of gestation. Blood samples were allowed to clot overnight, centrifuged at 3000 x g for 15 min, and stored at -18°C until analysis.

After parturition, milk production and fat, protein and somatic cells contents were recorded monthly by the Italian Breeders Association (control type A4), and total, 305-d and ME (mature equivalent) milk yield were then calculated, as well as total and 305-d fat and protein yield. Four heifers were eliminated during late gestation and/or early lactation due to different causes, and milk recording was carried out on 19. The number of cows, milk production at the first ten monthly recording days and the performance during the 1st lactation are in Table 1. The number of cows at the end of the lactation was strongly reduced due to managerial choice with reference to culling. This fact could have had slight repercussions on the results because the majority of eliminations occurred in very late lactation. For each heifer, the pedigree index for Total Economic Index (ITE) was obtained by the Italian Brown Cattle Breeders' Association, as well as the genetic indexes calculated as parent averages for milk, fat and protein production (kg) and fat and protein percentage.

Serum ß-LG levels were determined using a bovine ELISA test (Bethyl Laboratories, Inc. Montgomery, TX, USA). Briefly, 100 µl of anti rabbit-ß-LG (lot. A10-125A-7) were adsorbed in 96 well-plates for 60 min at room temperature. After four washings in a phosphate buffer, the wells were incubated with BSA 1% for 30 min for quenching. Following another four washings in a phosphate buffer, both the standard curve range from 250 ng/ml to 3.9 ng/ml (lot. RC10-125-4) and samples were incubated for 60 min. Therefore, after four additional washings, 100 µl of anti ß-LG conjugated with peroxidase (lot. A10-125P-4, 1/45.000) were added. Finally, 100 µl of substrate TMB (3,3',5'5-tetramethylbenzidine, SIGMA Chemical Co.) were incubated for 10-30 min. Optical density was measured by microspectrophotometer at 490nm (SLT, Spectra Milano, IT). Collected data were plotted in 4 parameter curves, r = 0.9.

The data concerning milk production, milk fat, protein and somatic cell content at each test day control and in the complete lactation and genetic indexes were analysed according to the least squares method (SAS, 2004), using the following model 1:

$$y_{ij} = m + M_i + \varepsilon_{ij}$$

where y = individual observations; m = overall mean; M_i = fixed effect of month at parturition (9 levels, from June to February); ε = residual error.

Due to unequal variances at different physiological stages, the data concerning serum ß-LG content during the late gestation period were previously transformed, using base 10 logarithms, and then analysed by means of the following model 2:

$$y_{ijk} = m + W_i + S_j + bX_{ijk} + \varepsilon_{ijk}$$

where y = individual observations; m = overall mean; W_j = fixed effect of sampling period (3 levels: 20th, 26th, 32nd week of pregnancy); S_j = random effect of sire (14 levels); b = regression coefficient with pedigree index for ITE (X_{ijk}); ε = residual error.

Residual values of $\log_{10}$ serum ß-LG content at different gestation times following the application of model 2 were submitted to Pearson correlation analysis with milk, fat, protein production (total and 305-d) and ME milk production in 1st lactation.

Linear and multiple (stepwise method) regression analyses were then performed in order to obtain prediction equations of production parameters from $\log_{10}$ serum ß-LG content and/or genetic indexes for milk, fat and protein production in first lactation.

Results and discussion

Number of cows involved in the trial, and LS means of milk production, fat, protein and somatic cells contents at each test day control during first lactation are in Table 1. Total and 305-d milk, fat and protein production, ME milk

Table 1. Number of cows, milk production, fat, protein and somatic cells contents in first lactation as related to monthly milk recording in IB cows (LS means ± SE).

Monthly milk recordings	n.	Milk production (kg)	Fat (%)	Protein (%)	Somatic cells (n.*1000)
1	19	25.4 ± 1.4	4.83 ± 0.27	3.41 ± 0.08	480 ± 256
2	19	26.2 ± 1.5	3.78 ± 0.15	3.56 ± 0.06	294 ± 109
3	18	26.4 ± 1.3	3.74 ± 0.15	3.67 ± 0.07	369 ± 149
4	18	24.9 ± 1.3	3.85 ± 0.21	3.66 ± 0.05	258 ± 80
5	18	23.3 ± 1.6	3.82 ± 0.19	3.65 ± 0.07	267 ± 94
6	17	23.2 ± 1.7	3.77 ± 0.19	3.66 ± 0.07	212 ± 98
7	16	23.0 ± 1.4	3.67 ± 0.18	3.62 ± 0.07	188 ± 74
8	14	20.9 ± 1.5	3.73 ± 0.17	3.69 ± 0.08	209 ± 88
9	11	20.8 ± 1.7	3.76 ± 0.25	3.81 ± 0.08	316 ± 196
10	9	18.9 ± 1.3	4.08 ± 0.25	3.99 ± 0.11	188 ± 92

IB: Italian Brown; LS: least squares.

production and prevision genetic indexes are in Table 2. The maximum milk production was 26.4 kg/d at month 3 of lactation and the production level (7891 kg) was 33.7% above the mean production of primiparous Italian Brown cows (5899 kg; AIA, 2002).

Least squares means for serum ß-LG content (±SE) at weeks 20, 26 and 32 of gestation in Italian Brown heifers were 706±78, 753±66 and 772±63 ng/ml, respectively (P>0.05). Individual values ranged from 96 to 1471 ng/ml. Mao and Bremel (1995) also reported a wide variation (from 16 to 1200 ng/ml) of serum ß-LG content at week 26 of gestation in Holstein heifers. Mammary growth accelerates throughout pregnancy, and this is fastest during the later stages of pregnancy (Forsyth, 1986; Tucker, 1987). Mao *et al.* (1991) described the development of mammary glands in Holstein heifers as a continuous exponential process throughout gestation that can be well described by the following equation: $\log_{10}$ ß-LG ng/ml = 1.29 * $e^{0.0053 * \text{day of pregnancy}}$. Our study on Italian Brown heifers confirmed the shape of the curve. The exponential equation, calculated from data at 20, 26 and 32 weeks of gestation is the following: $\log_{10}$ ß-LG ng/ml = 2.67 * $e^{0.0003 * \text{day of pregnancy}}$.

Correlations between residual values of $\log_{10}$

Table 2. Milk, fat and protein yields during 1st lactation in IB cows and prevision indexes for ITE, milk, fat and protein production (LS means ± SE).

	Milk (kg)	Fat (kg)	Fat (%)	Protein (kg)	Protein (%)
Total production	7891 ± 667	304 ± 26	-	285 ± 25	-
305 d production	8635 ± 460	286 ± 14	-	262 ± 12	-
ME production	7169 ± 367	-	-	-	-
Pedigree index for ITE	249 ± 28	-	-	-	-
Pedigree index	388 ± 21	12.8 ± 1.3	0.003 ± 0.011	10.9 ± 0.9	0.010 ± 0.007

Table 3. Correlation coefficients between residual values (following application of model 2) of log_{10} ß-LG content at week 20, 26 and 32 of gestation and production parameters in first lactation in IB cows.

	Week of gestation					
	20		26		32	
	r	P	r	P	r	P
Total milk production	-0.033	0.893	-0.024	0.922	0.455	0.050
Total fat production	0.070	0.777	-0.009	0.972	0.342	0.152
Total protein production	0.001	0.997	0.025	0.919	0.472	0.042
ME milk production	-0.226	0.351	-0.139	0.571	0.379	0.110
305 d milk production	0.116	0.658	0.128	0.623	0.315	0.218
305 d fat production	0.382	0.130	0.082	0.754	0.093	0.722
305 d protein production	0.090	0.732	0.152	0.562	0.395	0.116

serum ß-LG content at 20, 26 and 32 weeks of gestation and milk, fat and protein yield in the first lactation of Italian Brown cows are reported in Table 3. Serum ß-LG content at weeks 20 and 26 of gestation were never significantly correlated with production traits. At week 32 of gestation only total milk yield and total protein yield in first lactation were significantly correlated with serum ß-LG content during gestation (0.455 and 0.472, respectively; $P \leq 0.05$). Mao and Bremel (1995) reported significant correlations (0.456, 0.541, 0.479, respectively) between log_{10} serum ß-LG content at 26 weeks of gestation and 305-d milk, fat and protein production in primiparous Holstein cows.

A set of prediction equations of milk, fat and protein yields during first lactation from ß-LG content during pregnancy was then calculated with the aim of better estimating the role of a physiological marker, such as the ß-LG content

Table 4. Parameters (R^2, SE of the dependent variable, P of the regression) of the prediction equations of milk, fat and protein yields (kg) in first lactation from log_{10} serum ß-LG content during gestation (ng/ml) and/or parent averages genetic values in IB cows.

Dependent variable	log_{10} serum ß-LG content at week 32 of gestation (A)			Parent average genetic indexes (*) (B)			A+B		
	R^2	SE	P	R^2	SE	P	R^2	SE	P
Total milk yield	0.264	1919.4	0.024	0.135	2144.6	ns	0.296	1935.4	ns
Total fat yield	0.179	83.7	ns	0.093	90.7	ns	0.195	85.4	ns
Total protein yield	0.228	73.2	0.039	0.164	81.1	ns	0.243	74.8	ns
ME yield	0.234	1393.5	0.036	0.322	1351.6	0.045	0.472	1275.0	0.048
305-d milk yield	0.253	1090.6	0.040	0.421	993.9	0.022	0.488	969.4	0.029
305-d fat yield	0.244	47.2	0.044	0.543	41.0	0.039	0.577	39.5	0.026
305-d protein yield	0.232	36.9	0.050	0.454	33.4	0.043	0.392	34.0	0.039

ns: P>0.05.
(*) for milk, fat and protein production (kg) and fat and protein percentage, alone or in conjunction.

during first gestation. Due to the high correlation coefficients shown above, 32nd week $\log_{10}$ ß-LG content was chosen as an independent variable. The resulting linear equations generally gave a reliable prediction of the majority of the productive parameters, with determination coefficients ranging from 17.9% for total fat production to 26.4% for total milk production (Table 4). This could thus improve the selection of replacement heifers. Moreover, the precision of the predictive equations could be further improved by the addition of genetic information, such as the pedigree index for ITE or milk, fat and protein genetic indexes calculated as parent averages. As reported in Table 4, adding both ß-LG content in serum during the gestation (32nd week) and the parent average genetic indexes improved the prediction of total milk, fat and protein yields, ME and 305-d milk and fat production, as for 305-d protein production an improvement of the predictive ability was not shown. Also Mao and Bremel (1995) obtained a better prediction of milk production in first lactation by adding the ß-LG content at week 26 of gestation to parent average information (R^2 improved from 0.034 to 0.211 for milk yield; from 0.142 to 0.428 for fat yield and from 0.068 to 0.273 for protein yield).

Conclusions

The results suggest that ß-LG content at week 32 of gestation could be used as a physiological marker of milk, fat and protein production in first lactation in Italian Brown heifers, because of the high and significant correlation coefficients with some productive traits. Contrary to that observed by Mao and Bremel (1995) in Holstein heifers, the analysis of ß-LG content at an earlier stage of gestation in Italian Brown heifers was not useful, probably due to a different age at conception in our study and consequently to a later development of the mammary gland in Italian Brown heifers than in Holstein. A further hypothesis could refer to the different productivity level in the two breeds. It is also probable that the small number of observations could have played a role here. The association of parent average genetic indexes to ß-LG content could improve the prediction of future yield. In conclusion it is valuable to pre-estimate milk production in heifers in order to improve dairy productivity by means of a more accurate selection of replacement heifers using both genetic information and ß-LG content during gestation. Moreover, in the future the method could improve the accuracy in the genetic evaluation of bulls, and this trait could be included in animal models used in breeding to reduce environmental variation.

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