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# Relationship between serum $\beta$ -lactoglobulin content during gestation and reproductive efficiency in primiparous sows

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## ABSTRACT

The relationship between  $\beta$ -lactoglobulin ( $\beta$ -LG) serum concentration in sows during the last 8 weeks of gestation and subsequent piglet performance was investigated in 10 Dunel gilts. Two classes of gilts were identified with low (<150 ng/ml) or high (>150 ng/ml) average serum  $\beta$ -LG content. For both low and high content groups, equations were calculated to describe trends in serum  $\beta$ -LG content, respectively  $y_1=10.07e^{0.0237x}$  ( $R^2=0.3122$ ) and  $y_2=69.00e^{0.0201x}$  ( $R^2=0.6959$ ), where  $x$  is the number of days of gestation. Differences in serum  $\beta$ -LG content between the two groups were highly significant at all weeks ( $P<0.01$  from week 8 to 6 before farrowing;  $P<0.001$  from week 5 to farrowing). No significant differences ( $P>0.05$ ) between groups were shown for total number of piglets born, born alive, stillborn or mummified and piglet survival rates up until d 21 after farrowing. The group with high serum  $\beta$ -LG content during gestation showed higher litter weights at d 5 ( $P<0.05$ ) and d 21 ( $P<0.10$ ) and higher estimated milk production from farrowing to d 5 ( $P<0.10$ ). The results indicate that serum  $\beta$ -LG content during the final weeks of gestation could be used as an early indicator of reproductive efficiency, and that gilts with high content could be selected to improve herd productivity.

*Key words:* Sow,  $\beta$ -lactoglobulin, Reproductive efficiency, Litter

## RIASSUNTO

RAPPORTI FRA CONTENUTO DI  $\beta$ -LATTOGLOBULINA NEL SANGUE DURANTE LA GRAVIDANZA ED EFFICIENZA RIPRODUTTIVA NELLE SCROFETTE

*Lo scopo della ricerca, condotta su 10 scrofe primipare di ceppo Dunel, è stato quello di esaminare i rapporti esistenti fra il contenuto in  $\beta$ -lattoglobulina ( $\beta$ -LG) nel sangue nel corso delle ultime 8 settimane prima del parto e le performance della nidata durante lo svezzamento. Sono state identificate due classi di scrofe, una con basso contenuto (<150 ng/ml) ed una con alto contenuto (>150 ng/ml) di  $\beta$ -LG. Per entrambi i gruppi sono state calcolate equazioni che descrivono le variazioni nel corso della parte finale della gravidanza [risp.:  $y_1=10,07e^{0,0237x}$  ( $R^2=0,3122$ ) e  $y_2=69,00e^{0,0201x}$  ( $R^2=0,6433$ ), dove  $x$  rappresenta il n. di giorni di gravidanza]. Il contenuto di  $\beta$ -LG nel sangue delle scrofe è risultato diverso fra due gruppi in corrispondenza di tutto il periodo considerato ( $P<0,01$  dall'8ª alla 6ª settimana dal parto e  $P<0,001$  dalla 5ª al*

parto). Non sono emerse differenze significative ( $P > 0,05$ ) fra i due gruppi per quanto riguarda il numero totale dei suinetti nati, nati vivi, nati morti, mummificati ed i tassi di sopravvivenza fino a 21 d di età; le scrofe con alto contenuto di  $\beta$ -LG hanno mostrato nidiate con pesi maggiori a 5 d ( $P < 0,05$ ) e a 21 d ( $P < 0,10$ ) ed una maggior produzione latteica nei primi 5 d dopo il parto ( $P < 0,10$ ). I risultati indicano che il contenuto di  $\beta$ -LG nel sangue potrebbe rappresentare un utile indicatore della efficienza riproduttiva delle scrofe; quelle che presentano alti valori di  $\beta$ -LG nel corso della gravidanza potrebbero essere selezionate, contribuendo così ad una maggiore produttività dell'allevamento.

Parole chiave: Scrofa,  $\beta$ -lattoglobulina, Efficienza riproduttiva, Nidiata

## Introduction

One of the most important goals of swine breeding is to maximize sow productivity, so that an increase in the number of piglets born is also accompanied by an improvement in the ability to obtain weaned piglets (Kim *et al.*, 1999). To this aim, lactation performance is a major factor for sow productivity. In fact, sub-optimal mammary gland function is a limit to piglet growth potential (Boyd and Kensinger, 1998).

Sow milk is the principal energy resource for new-born piglets. The number of secretory cells in the mammary gland is the principal factor affecting milk production (Knight and Wilde, 1993). Previous studies have shown that the number of secretory cells at the onset of lactation could have a strong effect on milk production (Head and Williams, 1991) and a strong correlation between mammary gland DNA content and litter growth has been demonstrated (Nielsen *et al.*, 2001).

Gilts have a genetic potential for milk production at birth and udder development during growth affects milk production in the 1<sup>st</sup> lactation. A strong correlation between  $\beta$ -lactoglobulin ( $\beta$ -LG) content in blood serum during gestation and subsequent milk production in 1<sup>st</sup> lactation has been shown in heifers (Mao *et al.*, 1991; Mao and Bremel, 1995). Studies have also shown that  $\beta$ -LG in blood serum increases during gestation in correlation with the developing mammary gland of the heifer and that, because the secretory mammary cells are not held tightly together until just prior to parturition, the synthesized proteins can flow freely between cells and spill back into blood serum (Haenlein, 1995).

The aim of this study was to investigate if serum  $\beta$ -LG content during gestation could also be

a physiological marker of milk production in gilts, by determining its effect on reproductive efficiency and litter traits.

## Material and methods

The trial was conducted on 10 Duneil gilts of the same age and reared in similar environmental conditions during the prepuberal period. At the onset of the 3<sup>rd</sup> oestrus after puberty, gilts were artificially inseminated twice at 24 hour intervals using semen from one boar. Gestation was confirmed by echography on day 30 after insemination. Gilts were group-housed during gestation and fed a standard diet at the dose of 2.5 kg/head/day.

During the last 9 weeks of gestation, blood samples were collected from the jugular vein at weekly intervals. Serum  $\beta$ -LG content at week 9 from farrowing was considered as the basal content. Blood samples were allowed to clot overnight, then were centrifuged at 3000 x g for 15 min, and stored at -18°C until analysis.

Gilts were transferred to a nursery one week before farrowing and kept there until weaning. During lactation they were fed increasing doses of a standard diet (from 3.2 to 6.4 kg/head/day). The number of total born piglets, piglets born alive, dead or mummified and weaned piglets were recorded; litters were weighted at day 1, 5 and 21 after farrowing.

Serum  $\beta$ -LG levels were determined using a bovine ELISA test (Bethyl Laboratories, Inc. Montgomery, TX, USA), according to the manufacturer's instructions regarding cross reactivity with porcine  $\beta$ -LG. Briefly, 100  $\mu$ l of anti rabbit- $\beta$ -LG (lot. A10-125A-7) was adsorbed in 96 well-plates for 60 min at room temperature. After four wash-

ings in a phosphate buffer, the wells were incubated with BSA 1% for 30 min for quenching. Following another four washings in a phosphate buffer, standard curve ranged 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  $\mu$ l of anti  $\beta$ -LG conjugated with peroxidase (lot. A10-125P-4, 1/45.000) were added. Finally, 100  $\mu$ l of substrate TMB (3,3',5'5-tetramethylbenzidine, SIGMA Chemical Co.) were incubated for 10-30 min. Optical density was measured by microspectrophotometer at 490 nm (SLT, Spectra Milano, IT). Collected data were plotted in 4 parameter curves,  $r = 0.9$ .

Milk production from farrowing to days 5 and 21 was estimated according to the equations of Noblet and Etienne (1989).

Data concerning variations of serum  $\beta$ -LG content during the last 8 weeks of gestation were analyzed according to the least squares method (SPSS, 2002), using the following model:

$$\text{model 1: } y_{ijk} = m + W_i + L_j + WL_{ij} + bX_{ijk} + \varepsilon_{ijk}$$

where:

$y_{ijk}$  = individual observation;

$m$  = overall mean;

$W_i$  = fixed effect of week from farrowing (8 levels),

$L_j$  = fixed effect of class of average serum  $\beta$ -LG content (2 levels);

$WL_{ij}$  = interaction;

$bX_{ijk}$  = regression coefficient with basal serum  $\beta$ -LG content ( $X$ , ng/ml).

Non linear regression analysis was performed on the same data to generate prediction equations of serum  $\beta$ -LG content during the last period of gestation.

Data concerning reproductive efficiency and

litter traits were analyzed by least squares method using the following models:

$$\text{model 2: } y_{ij} = m + L_i + \varepsilon_{ij}$$

$$\text{model 3: } y_{ij} = m + L_i + bX_{ij} + \varepsilon_{ij}$$

where:

$y_{ij}$  = individual observation;

$m$  = overall mean;

$L_i$  = fixed effect of class of average serum  $\beta$ -LG content (2 levels);

$bX_{ij}$  = regression coefficient with litter weight at birth (kg) or mean individual weight at birth (g), respectively for litter weight and for mean weight at d 5 and 21.

## Results and discussion

The results of analysis of covariance conducted by applying model 1 are reported in Table 1. All fixed factors showed high significance ( $P < 0.001$ ), while the covariate was not significant ( $P > 0.05$ ). This may suggest that variations in serum  $\beta$ -LG content are rather independent of the initial serum content. The  $R^2$  coefficient for model 1 was high (0.931), therefore indicating its validity to explain variability of serum  $\beta$ -LG content. According to Dodd *et al.* (1994), the number of weeks from farrowing was highly significant. A preliminary analysis on individual data allowed us to obtain two classes of gilts (6 and 4 gilts, respectively) with different average serum  $\beta$ -LG content during the final weeks of gestation (low:  $< 150$  ng/ml and high:  $> 150$  ng/ml). Due to the lack of data in the literature concerning serum  $\beta$ -LG content in sows during first gestation, this finding was not easily explainable, if not by hypothesizing an individual effect due to genetic factors.

Table 1. Results of analysis of covariance for serum  $\beta$ -LG content during the last 8 weeks from farrowing in pregnant gilts (model 1).

Source of variation	DF	$\sigma^2$	P
Class of weeks from farrowing	7	25,044.646	<0.001
Class of average $\beta$ -LG content	1	120,776.580	<0.001
Interaction	7	16,651.446	<0.001
Covariate	1	10,304.474	0.076
Error term	61*	3,135.521	-

\*: 2 observations missed due to technical causes.

Table 2. Variations of serum  $\beta$ -LG (ng/ml) content during the last 8 weeks from farrowing in pregnant gilts (least squares means  $\pm$  SE).

Weeks from farrowing	Average serum $\beta$ -LG content		P
	Low	High	
8	75 $\pm$ 30	287 $\pm$ 12	<0.01
7	74 $\pm$ 13	264 $\pm$ 51	<0.01
6	77 $\pm$ 20	271 $\pm$ 17	<0.01
5	80 $\pm$ 14	415 $\pm$ 29	<0.001
4	99 $\pm$ 14	497 $\pm$ 19	<0.001
3	107 $\pm$ 13	503 $\pm$ 30	<0.001
2	111 $\pm$ 11	511 $\pm$ 46	<0.001
1	137 $\pm$ 12	516 $\pm$ 30	<0.001

Table 2 reports least squares means ( $\pm$  s.e.) of  $\beta$ -LG content in blood serum during the last 8 weeks from farrowing in gilts with low and high average content. Mean serum  $\beta$ -LG content was significantly different in the two groups ( $P < 0.01$  until week 6 and  $P < 0.001$  from week 5 to 1). Regardless of the group, mean serum  $\beta$ -LG content rose from week 8 to 1 before farrowing. The rise was regular both for the low content ( $y = 10.07e^{0.0237x}$ ; SE = 47.96 ng/ml;  $R^2 = 0.3122$ ), and for the high content group ( $y = 69.00e^{0.0201x}$ ; SE = 101.43 ng/ml;  $R^2 = 0.6959$ , where  $x$  is number of days of gestation). Our results disagreed only in part with those reported by Dodd *et al.* (1994). In fact, those Authors found  $\beta$ -LG values in blood serum from multiparous sows to be  $24 \pm 7$  ng/ml between week 17 and 7 from farrowing, then  $98 \pm 36$  ng/ml at week 5 and  $227 \pm 55$  ng/ml at week 4. In the last week before farrowing they found values  $> 2000$  ng/ml on the day of parturition. Differences with data reported in the present study could be explained with differences in analytical method, sow breed and parity order (age) of sows that could justify a different development of the mammary gland.

The results from the analysis of reproductive efficiency in gilts with low (80.63 ng/ml) or high (394.30 ng/ml) mean  $\beta$ -LG content ( $P < 0.05$ ) during the last weeks of gestation are reported in Table 3. No significant differences between the groups were found ( $P > 0.05$ ) on total born, born alive, dead, mummified, alive at 5 and 21 days, probably

due to the low number of gilts. The ratio between piglets alive at 5 or 21 days and total born was higher in the group with high  $\beta$ -LG content. Litters from high serum  $\beta$ -LG content group showed the highest total and individual weights at 5 ( $P < 0.05$ ) and, in part, at 21 days of age ( $P < 0.10$  for litter weight). The remark is very important because piglet survival during weaning is strictly dependent on their ability to intake feed and to grow. The advantage of piglets from high serum  $\beta$ -LG content group litters with respect to low content group litters, could be explained by higher estimated milk production of sows in the first days after farrowing ( $P < 0.10$ ). On the contrary, estimated milk production over the complete lactation period (21 days) was not significantly different between the groups ( $P > 0.10$ ), but mean values were higher in high serum  $\beta$ -LG content group.

## Conclusions

Milk production in gilts is not a primary trait, from the economic point of view, but a secondary trait, because it affects piglets' survival. This study, therefore, evaluated the effects of serum  $\beta$ -LG content on the reproductive efficiency of gilts. The observation of gilts with high average serum  $\beta$ -LG content during the last 8 weeks of gestation, together with the identification of gilts with low ( $\sim 80\%$ ) content, has not been previously reported in literature. Moreover, sows from the high serum  $\beta$ -LG content group had higher litter weights

Table 3. Effect of serum  $\beta$ -LG content during gestation on sow reproductive parameters and litter traits (least squares means).

Parameter		Serum $\beta$ -LG		SE
		Low	High	
Mean $\beta$ -LG content	ng/ml	80.63 a	394.30 b	60.35
Piglets:				
- total born	n.	11.25	10.33	2.22
- born alive	"	10.63	10.00	1.88
- dead	"	0.33	0.25	0.68
- mummified	"	0.38	0	0.94
- alive at d 5	"	9.13	9.00	1.45
- alive at d 21	"	9.00	9.00	1.63
- alive at d 5/total born	%	81.16	87.12	-
- alive at d 21/total born	"	80.00	87.12	-
Litter weight:				
- at d 0	kg	15.45	13.62	3.51
- at d 5	" (*)	20.42 a	24.02 b	2.04
- at d 21	" (*)	53.28 A	60.62 B	5.96
Mean weight:				
- at d 0	g	1456	1408	274
- at d 5	" (**)	2290 a	2531 b	152
- at d 21	" (**)	6111	6508	748
Estimated milk production:				
- from d 0 to d 5	g/d	516 A	636 B	87
- from d 0 to d 21	"	684	712	136

A,B =  $P < 0.10$ ; a,b =  $P < 0.05$

(\*) = covariated with litter weight at d 0

(\*\*) = covariated with mean weight at d 0

(+17%), higher mean piglet weights (+10%) and a higher estimated milk production (+23%) during the first 5 days after farrowing.

These preliminary results should be confirmed by studies on a higher number of animals. In that case, it could be suggested that monitoring gilts during gestation and selecting those with higher mean serum  $\beta$ -LG content for reproduction could improve herd productivity or, alternatively, could reduce costs by means of a reduction of the number of gilts for reproduction. This of course would mean a real potential in improving sow productivity.

*The paper must be attributed equally to Authors.*

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