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Serum Concentrations of Leptin, Ghrelin and Cortisol are not Direct Related to the Loss of Backfat Thickness, Body Weight, Protein and Fat at Weaning of Second Parity of Crossbreed Sows (Landrace x Large White)

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Abstract: The experiment was conducted to investigate the relationships between changes in body weight and backfat thickness, body protein and fat, serum hormones concentration and metabolites which are involved in the regulation of appetite and energy metabolism at weaning in 24 sec parity crossbreed sows (Landrace x Large White; L×LW). Samples were collected and divided into three groups: 48 h before Weaning (W-48 h), at Weaning (W), 48 h post-weaning (W+48 h). Leptin, ghrelin, cortisol, estradiol-17β, protein, lactic acid and glucose were determined in blood serum of sows. In addition, sow body chemical composition were estimated using two different equations. Using regression analysis association between leptin and ghrelin levels at W-48 h, W and W+48 h were estimated. The 4 weeks of lactation induced significant changes in decrease of Body Weight (BW) and Backfat Thickness (BFT) as well as in loss of body fat and protein. Both BW and BFT loss were strong positive correlated with the Weaning to Estrus Interval (WEI) with exception of evaluated sow body chemical composition which had non-significant relationships with WEI. Mean concentration of glucose, estradiol-17β, leptin and ghrelin did not differ between investigated groups. High level of cortisol is mostly related to the stress at weaning caused by piglet removal. In sow, no antagonistic relationships between leptin and ghrelin level were observed. Moreover, concentration of serum leptin, ghrelin and cortisol had no significant correlation with the changes in calculated sow body protein and fat and with reproductive performance after Weaning (WEI). In conclusion, this study suggest strong evidence that it is not likely direct one to one relationship between those hormones and changes in crossbreed (L×LW) sow body weight, fat, protein and backfat thickness.

Key words: Sow, lactation, metabolites, hormones, fat

INTRODUCTION

Selection in pig production has been focused to increase the number of functional glands and consequently litter size which is demonstrated with higher suckling demand and increase milk production from individual glands (Hartmann *et al*., 1997). During lactation sows produces high milk yields and they have low appetite. Insufficient feed intake during lactation results in directing to intense catabolism and therefore greater weight loss which has a negative impact on reproductive performance (Guedes and Nogueira, 2001). Excessive weight loss reduces the proportion of sows returning to estrus increasing the re-mating interval and decreases the rate of pregnancy (De Rensis *et al*., 2005). Regulation of appetite is due to the complex interaction between the gastrointestinal tract, adipose tissue and the appetite center in the hypothalamus which controls the intake of nutrients and the amount of energy stored in the form of fat depots. Lower feed intake is accompanied by higher lipid mobilization (Prunier *et al*., 2001) and loss of protein mass (Clowes *et al*., 2003) without detrimental influence on milk production. Induced feed restriction during lactation increased plasma cortisol level in sows due to need for greater lipolysis in these sows (Baidoo *et al*., 1992).

In adipose tissue is produced and secreted protein, recently discovered leptin. It is a 16 kDa protein consisting of 146 amino acids and secreted by adipocyte into blood stream in response to changes in body weight or energy (Zhang *et al*., 1994). It has been implicated in regulation of feed intake, energy metabolism, body composition and reproduction (Barb *et al*., 2001; Kuehn *et al*., 2009). Metabolic hormone leptin serve as a

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sign of positive energy balance and the long to medium-term regulator of energy homeostasis and body adiposity (Havel, 2002). More recently, another hormone ghrelin has been disclosed as mediator of the effects of nutrition and metabolism on reproductive process. This hormone signals the energy deficit and stimulates increase metabolic efficiency and thus acts as a short to medium-term regulator of energy balance in the organism (Dong *et al*., 2009). Leptin and ghrelin are considered as antagonists in their action on appetite and metabolism (Sirotkin and Meszarosova, 2010).

Gilts with high backfat subsequently had a shorter weaning to estrus interval, larger litter size and higher farrowing rate in comparison to gilts with lower backfat thickness (Barb *et al*., 2008). In addition, sows exhibited greatest amount of backfat thickness at farrowing and weaning had highest serum concentration of leptin (Estienne *et al*., 2000). Porcine leptin can alter lipid synthesis indirectly with inhibition glucose conversion to lipid and directly to inhibit fatty acid incorporation by porcine adipocytes (Ramsay, 2003). Evidence suggests that anaerobic metabolism of glucose to lactate does not stimulate leptin production (Havel, 2000). Moreover, glucose output by primary porcine hepatocytes was time and dose-dependently stimulated by ghrelin (Gauna *et al*., 2005) and it has been suggested that ghrelin play a negative role in glucose metabolism (Dong *et al*., 2009). It has been demonstrated that the reducing of feed intake accompanied with catabolism of body reserves are under control of numerous hormones during lactation with effect on weaning to estrus interval.

Therefore, the objective of the present study was tend to determine the relationships between changes in body weight and backfat thickness, body protein and fat, serum hormones concentration and metabolites which are involved in the regulation of appetite and energy metabolism at 48 h before weaning, at weaning and 48 h post-weaning in sows.

MATERIALS AND METHODS

Animals and housing: Experiment was conducted on 24 crossbreed sows (Landrace x Large White) in second lactation in a high-production commercial herd in Slovenia. During gestation sows were kept in group pens measuring 3.6×4.8 m (12 sows per pen, i.e., 1.25 m^2 per sow). Automatic feeding was carried out once per day in the morning. The feeding troughs were 60 cm long, 30 cm wide and 20 cm high. Artificial light (60lx) was provided. The sows had *ad libidum* access to water (automatic nipple watering).

Between days 109 and 112 of gestation the animals were moved into farrowing house equipped with individual farrowing crates where they remained during 28 days lasting lactation. The newborn piglets were cross-fostered 5-12 h after birth to give 12 piglets per litter, taking into account the number of functional teats, the number of piglets in previous lactations and the general condition of each sow. The size of a single farrowing crate was 2.4×1.6 m (3.84 m^2), floors were equipped with a plastic grate (1×5 cm mesh). One part of each farrowing pen was equipped with a special heating plate for piglets (150 cm long, 60 cm wide at one end tapering to 21 cm at the other end) along with the infrared light. Artificial light was provided (60lx). The feeding troughs for sows were 38 cm long, 40 cm wide and 20 cm high. Feed withdrawal was implemented on the day before of farrowing. At farrowing day each sow received 13.1 MJ ME/kg (16.2% crude protein, lysine 0.95%, tryptophan 0.19%) of fed, approx. 1 kg/day. Between days 1 and 7 of lactation sequential increases of fed was assembled and reached at day 7 about 65 MJ ME in 5 kg of fed divided into three meals. At day 9 of lactation fed was increased on 6 kg of fed or 79 MJ ME distributed into four meals. This feeding regime was unchanged until day 25 of lactation. Last 3 days of lactation was amount of fed reduced, i.e., at day 26 decreased to 65 MJ ME in 5 kg of fed, days 27-26 MJ ME in 2 kg of fed and last meal was at 15 PM. At day 28 of lactation the fed was withdrawal. Ambient temperature was maintained between 18-19°C.

Sows and pigs had *ad libidum* access to water (automatic nipple watering). Approximately 5 days after parturition, the piglets received pre-starter feed in special feeding troughs (16×34×14 cm).

After weaning sows were transferred to the service area, i.e., individual pens with an area of 1.92 m^2 (2.15×0.6 m) with concrete rifts. The sows were feed once daily at 5 AM using feeding machines that transferred the feed into the feeding troughs (60×30×20 cm). They received with exception of weaning day fed with 11.8 MJ ME/kg (13.0% crude protein, lysine 0.65%, tryptophan 0.16%). Total daily amount of fed per sow was 4 kg contained 46 MJ ME. 330 lx of artificial light was provided for 16 h/day. The sows were exposed to mature boar twice daily for estrus detection. The Weaning to Estrus Interval (WEI) was recorded.

This study was carried out in accordance with the European Union directive (86/609/EEC) that has been approved by the Slovenian government with Law Regulating the Protection of Animals.

Backfat and body weight measurements: Back fat thickness was measured using an ultrasound apparatus (Back-fat Scanner, Draminski, Poland). Measurements were conducted in 24 h after farrowing (BFT F) and at the day of weaning (BFT W). They were performed in the back area at the last rib, 5-8 cm lateral to the dorsal midline. Before the measurement, the selected area was

shaved, designated with color marker and oiled with paraffin oil. The designation of measurement area on each sow enabled us to perform every measurement at exactly the same point. The average of three successive measurements was taken (triple mode).

Body weight was individual determined in 24 h after Farrowing (BW F) and at Weaning (BW W) using special electronic scale for weighing (Meier-Brakenberg, Germany) with a display (Fancom B.V., F-Star 125, Netherlands).

Analyses and calculations: The difference between BFT F and BFT W was assigned as Backfat Thickness Loss (BFT L). The difference between BW F and BW W was evaluated as Body Weight Loss (BW L) during lactation.

Considering difficulties of direct measurements of body composition changes (Farrowing (F) and Weaning (W)) in sow Body Protein (BP) and Fat (BF) were estimated. First one proposed to Whittemore and Yang (1989):

$$BP(kg) = -2.3+(0.19\times Liveweight, kg)-(0.22\times Backfat P2, mm)$$

$$BF(kg) = -20.4+(0.21\times Liveweight, kg)+(1.5\times Backfat P2, mm)$$

and second equations according to Dourmad for estimation of body lipid and protein:

$$Lipid(kg) = -26.4+0.221\times EBW+1.331\times Backfat P2, mm$$

$$Protein(kg) = 2.28+0.178\times EBW-0.333\times Backfat P2, mm$$

Where:

EBW (kg) = The sow empty live weight estimated from the live Weight (BW) (EBW = $0.905\times BW^{1.013}$)

P2 (mm) = The backfat thickness at the level of last rib

The differences between characteristics estimated at farrowing and weaning were used for calculation as loss of specific tissue during lactation.

Analytical methods: From each animal, 9 mL of blood was collected 48 h before weaning, at weaning and 48 h post-weaning at 10 AM from vena cava cranialis dextra with 2.1×80 mm hypodermic needle (Bovi-vet®) into 20 mL syringe (Terumo® Syringe) during a brief nose snare restraint. Blood samples were drawn into chilled polypropylene tubes coated with K_3-EDTA, stored at 4°C for approximately 8 h and then centrifuged for 10 min (3000×g). Serum samples were transferred into fresh tubes and stored at -20°C before being analyzed. Prior blood collection rectal temperature was measured with digital thermometer and recorded.

In samples total protein, glucose and lactic acid content was determined by the LISA 200/ALIZE biochemical analyzer using reagents according to manufactures instructions (Biomerieux, France).

The level of hormones in serum was estimated by commercially available ELISA kits according to manufactures instructions. Serum concentration of ghrelin was determined using Human Acylated Ghrelin Enzyme Immunoassay kit (SPI-Bio, Bertin Pharma, Montigny le Bretonneux, France). The sensitivity of the method was 1.5 pg mL^{-1} and variability coefficients within and among samples were 7.9 and 8.3%, respectively. Leptin content in serum was determined by human leptin high sensitive ELISA (IBL International, Hamburg, Germany). Assay sensitivity was 2.13 pg mL^{-1}, intra and inter-assay coefficients of variation were 6.9 and 8.9%. Level of cortisol in serum was estimated using Cortisol ELISA (IBL International, Hamburg, Germany) with limit of detection at 0.005 μg dL^{-1}, intra and inter-assay coefficient of variation 6.8 and 8.1%. Concentration of estradiol-17β in serum samples was determined with 17β-Estradiol ELISA (IBL International, Hamburg, Germany). Analytical sensitivity was 9.714 pg mL^{-1} and variability coefficients within and among samples were 4.6 and 6.8%, respectively.

Statistical analyses: All data were analyzed using IBM SPSS Statistic Version 21. Results are expressed as mean±SEM. The variations of plasma concentration of hormones and metabolites at 48 h before Weaning (W-48 h), at Weaning (W) and 48 h post-weaning (W+48 h) were subjected to one-way Analysis of Variance (ANOVA). The significant difference between means were analyzed by the Duncan's test. To compare means for body weight, backfat thickness and estimated body chemical composition changes at farrowing and weaning the paired-samples t-test was used. Pearson's correlation coefficient was used to examine associations among body weight and backfat thickness at farrowing and weaning and weaning to estrus interval. Additional correlations were evaluated between leptin, ghrelin, cortisol, estradiol-17β and characteristics of estimated chemical body composition at three time points (W-48 h, W, W+48 h). Regression analysis was used to determine relationships between the levels of plasma leptin and ghrelin. A significant difference was in all tests declared at $*p<0.05$ and $**p\leq0.01$.

RESULTS AND DISCUSSION

At weaning had sows' significant lower body weight in comparison to farrowing (209.3±1.5 vs. 185.4±2.5 kg; p = 0.000). The similar significant reducing was documented for backfat thickness during lactation measured at farrowing and at weaning (17.31±0.35 vs. 12.18±0.45 mm; p = 0.000) (Table 1).

Calculated chemical composition of sow body according to Whittemore and Yang (1989) shown significant change between farrowing and weaning of estimated body fat (49.50±0.73 vs. 36.80±1.14 kg, p = 0.000) and protein (33.65±0.26 vs. 30.24±0.41 kg, p = 0.000). Similarly, using second equation for estimation of body chemical composition by Dourmad significant decrease at weaning regarding farrowing of body lipids (39.03±0.67 vs. 27.37±1.05 kg, p = 0.000) and body proteins (30.67±0.22 vs. 28.48±0.32 kg, p = 0.000) were noticed.

Mean protein values were at 48 h before weaning 87.3±2.0 g L^{-1}, at weaning 90.0±3.7 g L^{-1} and tended to increase at 48 h post-weaning to 96.0±2.0 g L^{-1} (p = 0.065). Mean lactic acid values were lowest at 48 h before weaning 2.38±0.20 mmol L^{-1}, increased at weaning to 2.83±0.23 and reached the peak at 48 h post weaning 3.11±0.17 mmol L^{-1} (p = 0.034). Mean glucose values were 48 h before weaning 4.65±0.17 mmol L^{-1}, increased at weaning to 4.90±0.19 mmol L^{-1} and returned at 48 h post-weaning to 4.47±0.15 mmol L^{-1} (Table 2).

Mean plasma estradiol-17β concentrations were 48 h before weaning 52.9±4.1 pg mL^{-1} and tended to increase at weaning to 61.0±5.0 pg mL^{-1} and thereafter 48 h post-weaning decreased to 57.5±4.4 pg mL^{-1}. Mean plasma cortisol concentrations were significant increased at weaning (39.1±4.0 ng mL^{-1}; p = 0.000) in comparison with 48 h before and post-weaning (19.2±2.7, 15.1±1.7 ng mL^{-1}). No significantly changes in mean concentrations of leptin (1525±5, 1518±6 and 1539±10 pg mL^{-1}; p = 0.129) and ghrelin (26.3±3.8, 25.8±3.9 and 26.8±3.7 pg mL^{-1}; p = 0.984) were observed at any time points (Table 2). Regression analysis demonstrated no relation between plasma leptin and ghrelin levels (Fig. 1).

Figure 1 shows the mean plasma leptin concentrations in blood samples obtained 48 h before weaning (a), at weaning (b) and 48 h post-weaning were plotted against the mean plasma ghrelin concentrations collected at the same time points. Coefficients of determination (R^2) were very low (0.007, 0.012 and 0.014) and consequently were very low were its quadratic root expressed as correlation coefficient's.

Table 1: Estimated body composition at farrowing and weaning of sows in second parities using two different equations[a, b]

Body composition	Farrowing	Weaning[1]	p-value
Body weight (kg)	209.3±1.500	185.4±2.500	0
Backfat thickness (cm)	17.31±0.35	12.18±0.45	0
Body fat (kg)[a]	49.50±0.73	36.80±1.14	0
Body protein (kg)[a]	33.65±0.26	30.24±0.41	0
Body lipids (kg)[b]	39.03±0.67	27.37±1.05	0
Body protein (kg)[b]	30.67±0.22	28.48±0.32	0

[1]Weaning at day 28 of lactation; [a]The body fat and protein estimated by the equation of Whittemore and Yang (1989); [b]The chemical composition of body calculated using equation of Dourmad

Table 2: Mean (±SEM) serum concentration of protein, lactic acid, glucose, estradiol-17β, cortisol, leptin and ghrelin, 48 h before Weaning (W-48 h), at Weaning (W) and 48 h post-weaning (W+48 h)

Parameters	W-48 h	W	W+48 h
Protein (g L^{-1})	87.3±9.6[a]	90.0±3.7[ab]	96.0±2.0[b]
Lactic acid (mmol L^{-1})	2.38±0.98[a]	2.83±1.12[ab]	3.11±0.83[b]
Glucose (mmol L^{-1})	4.65±0.85	4.90±0.93	4.47±0.72
Estradiol-17β (pg mL^{-1})	52.9±4.1	61.0±4.9	57.5±4.4
Cortisol (ng mL^{-1})	19.2±2.7[a]	39.1±4.0[b]	15.1±1.7[a]
Leptin (pg mL^{-1})	1525±5	1518±6	1539±10
Ghrelin (pg mL^{-1})	26.3±3.8	25.8±3.9	26.8±3.7

[a, b]Values signed with different letters are significantly different (p<0.05)

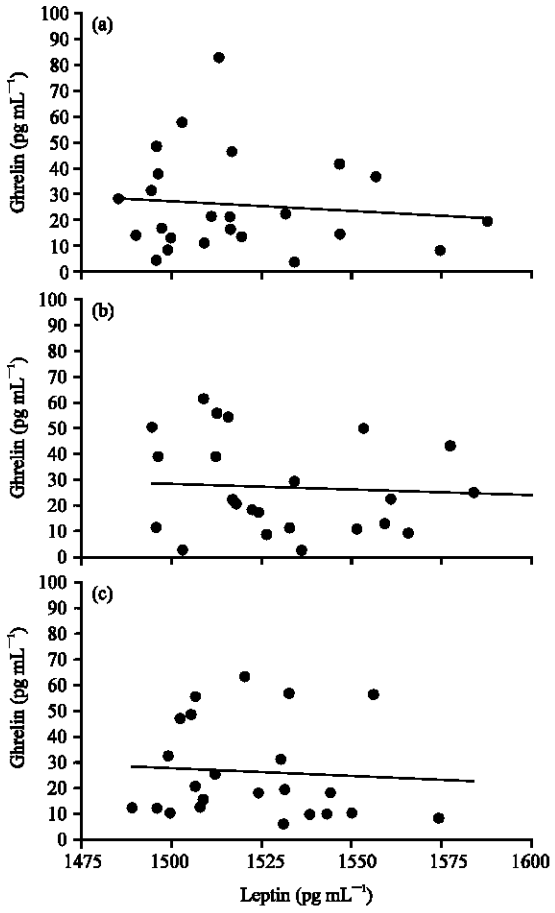

Fig. 1: The plasma leptin and ghrelin levels in blood samples collected 48 h. a) Before Weaning (W-48 h), b) at Weaning (W) and c) 48 h post-weaning (W+48 h)

There were no correlations between plasma levels of leptin and ghrelin. At all three time points parameter estimated as regression coefficients b1 was low and negative (-0.063, -0.077 and -0.042) and between plasma levels of leptin and ghrelin was no significant ($p>0.58$) relationships.

As documented in Table 3 both BW L and BFT L were correlated with WEI. Especially BFT L was significant related to prolongation of WEI ($r = 0.728$; $p\leq0.01$). BFT F was significant related to BFT W ($r = 0.905$; $p\leq0.01$) and BW W ($r = 0.741$; $p\leq0.01$).

In Table 4, no significant correlations were found between calculated chemical compositions of sows at weaning evaluated using two different equations and blood levels of protein, lactic acid, glucose, estradiol-17β, cortisol, leptin and ghrelin.

During lactation a body weight loss and backfat thickness decreased throughout the 4 weeks period. Body mass and backfat thickness were significantly reduced for 11.4 and 29.5%, respectively (Table 1). The current experiment showed that lactating sows with second parities entered in catabolic state. In other studies lasted lactation 23-27 days and backfat thickness loss was observed in primiparous sows 20.2% and for body weight loss only 3.3% while the multiparous (4th-7th parity) sows remained anabolic (Guedes and Nogueira, 2001). It has been documented that backfat thickness loss of more than 5 mm or 28.6% is typical for sows (average parity 3.9) with condition at farrowing assigned as fat (BF 25 mm) (De Rensis *et al*., 2005). As shown in Table 1, it was clearly demonstrated in present study that sows at second parity was much leaner (BF 17.3 mm) and had higher rate of backfat thickness loss during lactation. However, it has been suggested that weight loss represented the loss of fat, water and protein while backfat thickness loss represented only a loss of fat (Guedes and Nogueira, 2001).

Excessive negative balance presented with loss of body weight and backfat thickness had effect on sow primiparous reproduction performance (Clowes *et al*., 2003) and multiparous sows (Skorjanc *et al*., 2008). Several reports demonstrated opposite opinion, i.e., no relationships between backfat thickness and weight loss (Esbenshade *et al*., 1986) between percentage of total weight loss and Weaning to Estrus Interval (WEI) or percentage of backfat thickness loss (total or by periods) and WEI (Guedes and Nogueira, 2001) and no association between WEI and backfat thickness at weaning (De Rensis *et al*., 2005). In contrast to the previous observations, present study showed (Table 3) that increasing loss of Body Weight (BW L) and Backfat Thickness (BFT L) significantly affect prolongation of

Table 3: Correlation coefficients between reproduction characteristic and body weight and backfat thickness measurements at farrowing and weaning

	WEI	BW F	BW W	BW L	BFT F	BFT W	BFT L
WEI	1	-0.220	-0.540**	0.478*	-0.419*	-0.647**	0.728**
BW F	-	1	0.518**	0.085	0.478*	0.386	-0.039
BW W	-	-	1	-0.808**	0.741**	0.763**	-0.429*
BW L	-	-	-	1	-0.534**	-0.623**	0.474*
BFT F	-	-	-	-	1	0.905**	-0.302
BFT W	-	-	-	-	-	1	-0.679**
BFT L	-	-	-	-	-	-	1

WEI = Weaning to Estrus Interval (days); BW F = Body Weight at farrowing (kg); BW W = Body Weight at Weaning (kg); BW L = Body Weight Loss (kg); BFT F = Backfat Thickness at Farrowing (mm); BFT W = Backfat Thickness at Weaning (mm); BFT L = Backfat Thickness Loss (mm); *$p<0.05$; **$p\leq0.01$

Table 4: Relationships between sow estimated chemical composition at weaning and hormones concentration measured 48 h before Weaning (W-48 h), at Weaning (W) and 48 h post-weaning (W+48 h)

Parameters	WEI	BF W (kg)[a]	BP W (kg)[a]	Lipid W (kg)[b]	Protein W (kg)[b]	BF loss (kg)[a]	BF loss (%)[a]	Lipid loss (%)[b]	Lipid loss (kg)[b]	Protein loss (kg)[b]	Protein loss (%)[b]
W-48 h											
Estradiol-17β	0.246	-0.135	-0.164	-0.137	-0.162	-0.025	0.072	0.079	-0.017	-0.002	-0.005
Cortisol	0.173	0.144	0.006	0.141	-0.035	-0.006	0.001	0.018	0.002	0.172	0.166
Leptin	-0.045	0.149	0.120	0.149	0.104	0.006	-0.053	-0.059	0.010	0.048	0.024
Ghrelin	0.327	-0.063	0.136	-0.059	0.187	-0.163	-0.069	-0.069	-0.173	-0.344	-0.341
W											
Estradiol-17β	0.039	0.145	-0.003	0.139	-0.047	-0.151	-0.124	-0.106	-0.141	-0.068	-0.077
Cortisol	0.377	-0.175	-0.128	-0.175	-0.108	0.028	0.132	0.148	0.033	0.052	0.052
Leptin	-0.214	0.112	0.081	0.112	0.067	-0.024	-0.092	-0.099	-0.016	0.033	0.011
Ghrelin	0.260	-0.120	-0.066	-0.121	-0.047	-0.133	-0.042	-0.032	-0.138	-0.137	-0.123
W+48 h											
Estradiol-17β	0.175	0.087	0.072	0.085	0.064	-0.145	-0.093	-0.088	-0.142	-0.273	-0.277
Cortisol	0.286	-0.153	-0.243	-0.158	-0.256	0.219	0.246	0.254	0.228	0.260	0.246
Leptin	-0.149	-0.060	-0.900	-0.060	-0.094	0.068	0.035	0.037	0.074	0.089	0.076
Ghrelin	0.096	-0.094	-0.066	-0.096	-0.054	-0.047	0.001	0.013	-0.050	0.048	0.055

[a]The body fat and protein estimated by the equation of Whittemore and Yang (1989); [b]The chemical composition of body calculated using equation of Dourmad *et al*. (1997); WEI = Weaning to Estrus Interval (days); BF W(kg) = Body Fat at Weaning (kg); BF Loss (kg) = Body Fat Loss (kg); BF Loss (%) = Body Fat Loss (%); BP W (kg) = Body Protein at Weaning (kg); Lipid W (kg) = Body lipid at weaning (kg); Protein W (kg) = Body protein at weaning (kg); Lipid loss (%) = Body lipid loss (%); Lipid loss (kg) = Body lipid loss N (kg); Protein loss (%) = Body protein loss (%); Protein loss (kg) = Body protein loss (kg); W-48 h = 48 h before weaning; W = Weaning; W+48 h = 48 h post-weaning

Weaning to Estrus Interval (WEI). Moreover, sows at weaning with higher BW W and BFT W had significantly shorter WEI interval. Both characteristics, BFT L and BW L were in positive significant relationships'. The present study provided the most direct evidence that losses of BFT and BW during lactation are related to longer WEI.

It has been reported that the level of leptin was correlated with the backfat thickness and/or feeding regime during gestation. In addition, as possible effects on circulating leptin concentration gender (barrows, gilts) or genetics (fat or lean breed, maternal and paternal lines) were suggested. They demonstrated positive relationships between the leptin concentration and the bakcfat thickness for six breeds (Berkshire, Chester White, Duroc, Landrace, Poland China and Yorkshire) (Berg *et al*., 2003). Quite similar results regarding the effect of genetic line of swine were drawn by other researchers. Despite the observed differences between breeds in previously studies, no positive correlation was found between blood leptin concentration and amount of subcutaneous fat in Polish Large White, Polish Landrace and line 900 (Mackowiak *et al*., 2004). In present study no relationships were found between leptin concentration and BFT and BW at weaning or loss of BW and BFT during lactation. A possible explanation may be that sows from the current study were crossbreeds L×LW selected for lean production. This suggestion is supported by findings that this type of animals could have lower level of leptin concentration in comparison with breed, i.e., Berkshire considered as breed with more fat. The Berkshire-sired pigs had greater levels of leptin in serum in comparison with Hampshire-sired pigs but there were no gender effect established on leptin concentration (Alberti *et al*., 2009). Interestingly, in study of the effect of feed restriction on hormones in immunocastrated pigs (Large White x Landrace) x Pietrain) was shown a significant lower leptin serum concentration at age 154 in entire males in comparison to surgical castrates fed *ad libitum* (Batorek *et al*., 2012).

Serum concentration of leptin at farrowing and weaning were highest in sows exhibiting the greatest amount of backfat (Estienne *et al*., 2000). Plasma leptin concentration was significantly higher in FAT (>24 mm) group in comparison to other two (MEDIUM (18-24 mm) and thin (<16 mm)) but no relationships between plasma leptin concentration and WEI were recorded (De Rensis *et al*., 2005). A level of leptin concentration could change during lactation. As has been previously observed leptin concentration increased between a day before weaning and day post-weaning in protein-restricted heavy sows (Quesnel *et al*., 1998). In contrast to previous findings in the present study, no significant differences in leptin concentration were noticed between W-48 h and W+48 h (Table 2). Restricting feed intake at weaning in present study may result in low leptin concentrations. These findings are supported with facts that restricting feed intake, i.e., in gilts was demonstrated with decreased leptin concentrations (Mao *et al*., 1999; Whisnant and Harrell, 2002). Refeeding restored in only 4 h leptin concentration to the level of full fed gilts (Whisnant and Harrell, 2002). Interestingly, in current experiment 2 days post-weaning no significant increase of plasma leptin concentration was noticed. A possible explanation is that lean sows in second parity had so great BW and BFT loss at the end of lactation and therefore need longer time for recovering to reach appropriate body condition. Sows fed the high-fiber diet (11% of crude fiber) during gestation had lesser concentration of leptin before farrowing than control sows and had greater appetite during lactation due to decreased secretion of leptin (Quesnel *et al*., 2009).

Leptin and ghrelin have been suggested as functional antagonist in terms of their effects on food intake and energy expenditure as well as in the long-term control of body weight. Their circulating levels fluctuate in a reciprocal manner and conduct opposite biological actions where leptin is a signal for energy abundance and ghrelin as a signal for energy insufficiency (Tena-Sempere, 2007). Inverse relationship between these two hormones was established recently in dogs after methylprednisolone administration (Yilmaz *et al*., 2007), rodents (Sanchez *et al*., 2004) and humans (Williams and Mobarhan, 2003). It was demonstrated that ghrelin was involved in a serial biological functions including regulation of food intake, body weight, gastrointestinal motility, hormone secretion (GH), glucose release, cardiovascular function, enzyme release, cell proliferation and reproduction of pigs (Dong *et al*., 2009). To the knowledge, present study for the first time tend to determine changes in concentration of ghrelin during lactation in lean type of sows. The current study indicated no differences at W-48 h, at W and W+48 h in concentrations of ghrelin (Table 2). Furthermore, at all three time points no relationships between leptin and ghrelin concentration was observed (Fig. 1). Researchers may assume that ghrelin has no important role on regulation of feed intake and consequently maintaining energy demands in lactating sows of lean type of crossbreeds (L×LW). Findings from present study support observation that ghrelin concentration is not associated with leptin level during sow lactation. Moreover, these results strongly suggest that between

these two hormones is no functional antagonistic relationship established during lactation. Contrary to the results, Iberian sows with predisposition to obesity which failing to display post-weaning estrus demonstrated a high decrease in backfat mass during lactation and lower backfat depth at weaning and those animals had lower levels of leptin and higher levels of ghrelin at weaning (Torres-Rovira *et al*., 2011). The difference between our and Govoni *et al*. (2007) and those results of Iberian sows are probably due to the genetic differences. Indeed, profound differences were in prepubertal plasma leptin levels between gilts of obese (Iberian) and lean genotype (LW×L) were shown (Gonzales-Anover *et al*., 2012). Iberian sow has great capacity for accumulation of subcutaneous and intramuscular fat and therefore has higher secretion of leptin (Torres-Rovira *et al*., 2011).

The largest energy store of the body is adipose tissue. Hepatic energy stores such as glycogen is activated for satisfying short-term needs and adipocytes are involved to maintain stores of triglyceride for the long-term energy requirements (Loftus, 1999). Gauna *et al*. (2005) indicated that glucose output by porcine primary hepatocytes was time and dose dependently stimulated by acylated ghrelin and inhibited by unacylated ghrelin. These findings suggested that ghrelin in pigs is likely to play a negative role in glucose metabolism (Dong *et al*., 2009). In present study no significant differences were observed in glucose levels between three investigated periods (Table 2). Weaning alone had no effect on glucose concentration. Moreover, no significant relationships were observed also between any calculated chemical body tissue (fat, protein) changes induced during lactation and investigated levels of hormones (Table 4).

In present study, the concentration of cortisol at weaning was significant higher in comparison to W-48 h (suckling) and W+48 h (no suckling) (Table 2). It is obvious that the cortisol concentration is mostly related to the stress at weaning. Stress caused by piglet removal on the day of weaning induced approx. The 2.0-2.6 fold increases of cortisol concentration. These results are in a good agreement with earlier observation not only for cortisol concentration but for glucose as well (Quesnel *et al*., 1998). They found the same shape and similar extent of increase of cortisol concentration in sow with low feed level during lactation. Nevertheless, reduction of fed or delayed meal had a weak influence alone on cortisol concentration (Merlot *et al*., 2011).

CONCLUSION

The knowledge of the mechanisms that regulate metabolism and appetite may help to increase feed intake in lactation. This will maintain animals in a good condition and will reduce the weight loss and possible risk of weaning to conception reproductive problems in sows. While there are some studies suggested relationships between cortisol, leptin, ghrelin and body fat tissue in the research no such connection was established. It is obvious that a sow body has a complex system of hormones that interact in a different ways. It is not likely a simple direct one-to-one relationship between those hormones and sow body weight.

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