Effect of Protein Restriction in Sows during Gestation and Lactation on Visceral Organ Mass and Serum Hormones Concentration in the Offspring

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ABSTRACT: In the pig, under-nutrition in utero causes low birth weight, a decrease in muscle fiber number, and a reduction in postnatal growth rate. Low protein intake during gestation and lactation has been demonstrated to increase the loss of body protein and to reduce the visceral organ mass of the newborn piglets. The effect of under-nutrition of pregnant swine on subsequent serum hormones characteristics of their offspring was investigated. The trial involved 16 pregnant MeiShan sows. The swine were allocated randomly to one of two groups: a treatment group (n=8) and a control group (n=8). During gestation and lactation, the diet of the treatment group was decreased to 50% of their daily protein requirement. The control group was fed 100% of their daily requirement to support the swine growth throughout gestation and lactation. All of swine were artificial insemination mated. Diets provided similar amounts of metabolizable energy. In this experiment, organ weights of piglets were reported as fresh weight (grams), scaled to empty body weight (EBW; grams per kilogram). Serum hormones of offspring were sampled at 0 d, 15 d and 35 d of lactation. Piglets were slaughtered at 0 d and 35 d of lactation for organ weight determination. The objectives of the current experiment were 1) to determine whether protein restriction alters levels of somatotropic hormones, insulin, insulin-like growth factor-1, thyroid hormones, and 2) to evaluate the relationship between these eventual alterations and visceral organ mass in piglets. During lactation, dietary protein restriction decreased liver, spleen and kidney mass ($p<0.01$). But dietary protein restriction did not affect heart mass ($p>0.05$). Reduced protein intake decrease mean and basal plasma GH in piglets at 0 d, 15 d and 35 d ($p<0.01$). Mean insulin and insulin-like growth factor-1 concentrations were similar in both groups during lactation ($p>0.05$). Mean and basal plasma T_3 were higher ($p<0.01$), whereas plasma T_4 were lower in control group than in treatment group piglets on day 0. Mean T_3 concentration on day 15 and mean T_4 on day 35 were very significantly higher than control group. In conclusion, these results provide evidence that protein restriction throughout gestation and lactation alters circulating concentrations of hormones and has a negative impact on visceral organ mass.
[World Rural Observations 2009;1(2); 77-86]. ISSN: 1944-6543 (print); ISSN: 1944-6551 (online).

Key words: Maternal undernutrition; piglets; visceral organ mass; serum hormones

Abbreviation Key: BW = body weight; WW = weaning weight; LWB = litter weight at birth; ADG = average daily gain; MF = muscle fiber; LS=litter size; EBW=empty body weight; L sows=low protein intake sows; C sows= control group sows

1. INTRODUCTION

During gestation and lactation, voluntary feed intake of highly prolific is frequently inadequate to meet nutrition requirement for maintenance and milk yield, and these sows must mobilize fat and protein reserves (O'Grady et al., 1985; Noblet et al., 1990). Low protein intake has been demonstrated to increase the suckling piglet body protein loss and reduce visceral organ mass. There is increasing evidence that nutrition and changes in metabolic state influence the organ weight and associated metabolic hormones, including insulin, IGF-□, GH, and thyroid hormones(for review, see Schams et al., 1999; Prunier and Quesnel, 2000). However, much emphasis has been given to insulin and IGF-□, and less attention has been paid to GH, T_3 and T_4. It is widely accepted that insulin and somatotropic hormones (GH and IGF-□) play a key role in the regulation of fat and protein metabolism in the sow's gestation and lactation ,also has the effect on piglets' organ weight. The aim of the present experiment was to test the following hypotheses:1) low protein intake in sows induce alterations in insulin , somatotripic and thyroid hormones of suckling piglets, and 2) such alterations are associated with changes in visceral organ mass of the newborn piglets and ratio of organ mass : empty BW.

2. MATERIALS AND METHODS

2.1 Animals and Experimental Design

The experiment was conducted in two groups on a total of 16 MeiShan gilts. They were inseminated at 220±10 d of age and 130±8 kg weight in live. Gilts were moved from the gestation to the farrowing rooms on d 104±3 of gestation and were kept in individual farrowing crates in a building maintained between 20 and 25℃. When necessary, parturition was induced by an i.m. injection of 5 ml of cloprostenol on d 114 of gestation. Within 48 h after birth, litters were standardized to 10 piglets and choose two piglets to slaughter for sample. At the end of lactation, choose two piglets with similar weight to slaughter for sample. Water was freely available for the sows and the piglets throughout the experimental period.

During gestation, all sows were fed a diet containing 13.10Mcal of DE/kg, the protein in the diet of the treatment group was decreased to 50% of their daily requirement until term. The control group was fed 100% of their daily requirement to support the swine and allow for fetus growth throughout gestation (Table 1). After farrowing, sows were allowed to consume 2.5kg/d of the lactation diet. During lactation, the lactating swine were fed the same diet during pregnancy with groups. All of this feed allowance was calculated to meet 110% of the energy requirements of gestation and lactation. One day after farrowing, lactating primiparous sows were assigned within replicate to either a control (C: 14%CP) or a low (L:7%CP)-protein diet (Table 2). Diet provided similar amounts of DE (13.10Mcal/kg; Table 2).

Table 1 Ingredients and composition of sows during gestation (air-dry basis, %)

Ingredients	control group	experimental group
Corn	58.00	52.80
Soybean meal	12.00	0.00
Wheat bran	15.00	11.00
bone meal	1.00	0.50
Cyano-bran	0.00	0.00
$CaHPO_4$	0.00	0.70
Limestone	0.00	3.00
Corn sugar	10.00	27.00
Crude fiber powder	0.00	1.00
Premix	4.00	4.00
Total	100.00	100.00
Nutrient levels		
DE（MJ/Kg）	13.10	13.10
CP	12.10	6.10
CF	2.7	2.3
Ca	1.2	1.2
AP	0.4	0.4

Vitamin premix provided the following per kilogram of complete diet: VA, 12000IU； VD, 1600IU； VE,62IU； VK, 3.5mg； VB1, 1.2mg； VB2, 3.6mg； VB12, 0.015mg； nicotinic acid, 16mg； pantothenic acid, 12mg； folic acid, 1.55mg； Fe, 120mg； Cu, 22mg； Zn, 120mg； Mn, 42mg； Se, 0.18 mg； I, 0.2mg

2.2 Measurements and Sampling

Piglets were weight at 0 d, 15 d and 35 d of age. On the same day, feeding troughs were emptied in order to facilitate serial blood sampling. Injector was inserted into the right jugular vein of the piglets to assemble blood; piglets were deprived of feed 16 h before sampling. Blood samples were collected in heparinized tubes, immediately placed on ice, and centrifuged within 15 min for separation of plasma. Plasma samples were stored at -20℃ until assay. Two piglets of each litter were chosen at birth and at 35 day of age to slaughter for organ sample collection. The piglet was eviscerated and organ weights recorded. The liver, heart, kidneys, and spleen was dissected out of the visceral tissues and weight. The weight of the carcass, including hide and head, was defined as the eviscerated BW.

2.3 Analyses

2.3.1 Hormone assays. Plasma concentrations of insulin, GH, IGF-Ⅰ,and thyroid hormones were determined in duplicated using validated RIA (Louveau et al., 1991;and Camous et al., 1985), which was previously validated in the porcine species (Qian et al., 1999). Purified porcine hormones were radioiodinated by reaction with ^{125}I in the presence of chloramine-T (catalog number FH-408). Each serum sample was assayed in duplicate. A purified porcine reference standard was used to express the results.

For insulin, the intra- and interassay CV were 10% and 15% at 60μIU/ml, respectively, and the average sensitivity of assay, defined as 90% of total binding, was 1.5μIU/ml. For GH, the intra- and interassay CV were 9% and 13% at 5ng/ml, and the average sensitivity was 0.5ng/ml. For thyroid hormones, the intra- and interassay CV were 10% and 15%. Plasma IGF-Ⅰconcentrations were determined after an acid-ethanol extraction. The intra- and interassay CV were 7.4% and 17% at 258ng/ml, respectively, and the average sensitivity was 7.5ng/ml.

Table 2 Ingredients and composition of sows during lactation (air-dry basis, %)

Ingredients	control group	experimental group
Corn	61.00	56.00
Soybean meal	17.00	0.00
Wheat bran	12.00	15.00
bone meal	1.00	0.50
Cyano-bran	0.00	0.80
$CaHPO_4$	0.00	0.70
Limestone	0.00	0.00
Corn sugar	5.00	22.00
Crude fiber powder	0.00	1.00
Premix	4.00	4.00
Total	100.00	100.00
Nutrient levels		
DE（MJ/Kg）	13.10	13.20
CP	14.00	7.00
CF	2.8	2.7
Ca	1.2	1.2
AP	0.4	0.4

Vitamin premix provided the following per kilogram of complete diet: VA, 12000IU；VD, 1600IU；VE,62IU；VK, 3.5mg；VB1, 1.2mg；VB2, 3.6mg；VB12, 0.015mg；nicotinic acid, 16mg；pantothenic acid, 12mg；folic acid, 1.55mg；Fe, 120mg；Cu, 22mg；Zn, 120mg；Mn, 42mg；Se, 0.18 mg；I, 0.2mg

2.3.2 Organ mass Assays. Tissue masses are reported as fresh weight as previous data has suggested little difference in fresh and dry weights (Jin et al., 1994; Swanson, 1996; Swanson et al., 2000). Empty BW was the summed weight of the carcass (including head, skin and feet), heart, spleen, kidneys, liver. Empty BW is often used to evaluate the contribution of organ mass to metabolism of animals of different body sizes (Sainz and Bently, 1997; Koong et al., 1985). However, when considering nutrition delivery by pregnant animals, the uterine compartment should be considered metabolically active tissue and was, therefore, included in EBW (Rattray et al., 1974; Robinson et al., 1978). Total internal organ mass was the summation of the spleen, heart, liver, kidney.

2.3.3 Calculations and Statistical Analyses. Data were analyzed by ANOVA using the DUNCAN procedure of SAS (8.0). Sixteen lactating primiparous sows were allocated to this experiment. The data from sixty-four piglets of these sows were used for statistical analyses of hormones and organ mass. All data for piglets BW, EBW and organ mass, insulin, GH, IGF-Ⅰ, T_3, T_4 were analyzed using repeated measures in DUNCAN procedures. Litters were the experimental unit and significant differences among treatments were determined using sow. Time effect was week effect for litter, day effect for piglets' performance and pre-prandial and mean hormonal concentrations or sampling time effect for hormone profiles. In the results, the least squares means and the standard errors for least squares means are given.

2.4 Results

2.4.1 Organ mass

Dietary protein restriction did not affect BW, fetal number or total fetal mass of newborn piglets (Table 3). Heart weight was not affected by dietary treatment ($p>0.05$); however, mass of individual fetal mass was decreased ($p>0.05$). Spleen, kidney, and liver weights were decreased in restricted compared with piglets' of control group ($p<0.01$).

Liver weights were decreased as a result of maternal dietary protein restriction ($p<0.01$). When scaled to EBW, liver weights were also decreased comparing with the newborn piglets of control groups, but did not remain to 35d of lactation. Spleen mass was also decreased owing to dietary restriction from 0d to 35d ($p<0.01$), but when scaled to EBW, there is no significant differences on d0 of lactation, but on d 35. Kidney mass was decreased in response to maternal dietary protein restriction but in its ratio to EBW, it shows no significant difference either on d0 or on d35 ($p>0.05$). As a result of dietary restriction, heart mass was unaffected on d0 or d35. When scaled to EBW of piglets, it was unchanged ($p>0.05$). Dietary protein restriction was effective in reducing total internal organ mass. Owing to maternal undernutrition decreased in its ratio to EBW.

2.4.2 Hormone concentrations

Mean insulin, characteristics of GH profiles, mean IGF-Ⅰconcentrations are presented in Table-4. Plasma INS and IGF-Ⅰconcentrations did not differ among treatment groups and control groups at d 0, 15, and 35 ($p>0.05$). Mean insulin concentrations were higher in C groups than in T groups ($p>0.05$). Plasma concentrations of IGF-Ⅰare presented as Figure 1. The IGF-Ⅰconcentration was higher in C group piglets on day 0 and 35, but on the day 15, it was lower in C groups ($p>0.05$).Both of them were no difference between groups. Plasma concentration of GH was higher in treatment group than in control group piglets at d 0, 15, and 35. Representative profiles of plasma GH on day 0, 15 and 35 are illustrated in Figure 2. Concentrations of GH fluctuated at a high level during the lactation. Plasma mean and basal GH concentrations were higher in control groups. It decreased in treatment groups, but increased in control groups during lactation. Mean serum T_3 of control groups was greater on d 0 and 15, but similar with treatments' on d 35 ($p<0.01$). There was no difference between sample of piglets which from treatment groups and control groups. The number of T_3 on day 0, 15 and 35 of lactation was increased for piglets from treatment litter. From results reported in the recent literature (Table 5), in piglets from control gilts, it produced significantly higher plasma concentrations of the total T_3 for the entire test period. There were no differences between the piglets from control group and treatment group in circulating plasma concentrations of total and free T_4 on d0 and d15 of lactation, but on d35, T_4 increased greater in the piglets from control gilts. Mean T_4 of piglets from control gilts has a significant difference with treatment groups' ($p<0.01$).

2.5 Discussion

2.5.1 Visceral organ mass

We investigated the hypothesis that individual visceral organ weights are responsive to dietary protein restriction. Previous reports have evaluated the response of total visceral organ mass resulting from nutrition restriction during pregnancy (McNeill etal., 1997). Alternatively, the liver and other organs have been the focus of experiments (Robinson et al., 1978). The level of dietary restriction imposed on d 30 of gestation in the present trial. This level of restriction resulted in organ weights losses. In the present description of maternal system, piglets BW were decreased during lactation as a result of dietary restriction. Carcass of piglets was also decreased at d 0 and d 35, whereas scaled to EBW was decreased only at d0 or d 35. Dietary restriction from d 30 to d 114 of gestation did not change piglets' heart weight. There is no changes were observed when heart weights were scaled to EBW. In contrast, the difference was increased when kidney mass scaled to EBW. These observations indicate the necessity of the maternal tissues to maintain a threshold of functional mass to support the increased metabolic needs of conceptus development.

Spleen mass was decreased owing to restriction,

and the spleen has been shown to decline in mass as a consequence of advancing gestation with no change in blood flow. A similar lack of response in the spleen was also observed in a comparative serial slaughter trial (Heap and Lodge, 1967). In this experiment, the spleen was smaller owing to gestation and lactation dietary protein restriction. When scaled to EBW, the ratio did not show a decrease on d 0,but spleen rate decreased greater when compared with piglets' that belongs to litter of control group on d 35.

The liver plays a pivotal role during adaptation of maternal metabolism to pregnancy, which is reflected through changes in mass and function. After adjustment of organ weights for slaughter weight, liver weight was greater in piglets suckling control gilts. It has been suggested that the extrauterine deposition of N in sows fed high protein diets during pregnancy is stored as protein in muscle and other tissues; and is available as labile protein for use in lactation. However, there is also an effect on the fetus, for liver size and liver weight. When dietary restriction was imposed in this experiment, results showed a decrease in liver mass of piglets in response to protein restriction. A similar response was present when liver mass was scaled to EBW. This may also improved, that liver mass was sensitivity to nutrition demand.

The amount of protein in the gestation diet is not an important factor in affecting litter size or individual pig birth weight. Protein restriction during gestation appears to have a large effect on subsequent milk production, as measured by survival rate and litter weaning weight, indirectly have an effect on piglet's visceral organ mass. Why the size and gross composition of newborn pig is refractory to maternal dietary protein intake. Additional research is needed to further define total visceral responses to metabolic demands of pregnancy.

2.5.2 Hormone concentrations

Reduction in the protein content in the gestation and lactation diets resulted in a decrease in plasma insulin concentrations of piglets. Jones and Stahly reported that low protein intake during lactation reduced milk nutrition output, and overall litter growth in primiparous sows. This finding may explain the difference between groups in plasma insulin, a lower intake of protein attributed to the amount of milk production, to result to low insulin concentrations of the suckling piglets.

The reduction of plasma IGF-Ⅰ concentrations in response to protein restriction is consistent with previous results in the newborn piglets. The levels of IGF-Ⅰ did not change in response to maternal protein restriction. Under normal physiological conditions, IGF-Ⅰ secretion by hepatic and nonhepatic tissues is stimulated by GH. In L sows, low IGF-Ⅰ levels are associated with high plasma GH. It indicates an uncoupling between IGF-Ⅰ and GH. As suggested by Thissen et al. (1994) and Breier (1999), this uncoupling could be due to a hepatic resistance to GH itself, which is related to piglets low plasma insulin.

Irrespective of treatment, weaning induced marked changes in sow endocrine and metabolic regulations. Plasma GH concentrations and pulse frequency were higher during late lactation than after weaning, due to the neuroendicrine stimuli elicited by piglets during suckling (Rushen et al., 1993).Our data confirm that low protein intake reduced GH pulses of the suckling piglets during mid- and late lactation. GH resistance could have facilitated mobilization of lean tissue in the piglets suckling L sows. Similarly, differences in piglet plasma IGF-Ⅰ concentrations were no longer significant by day 0, 15, 35 of lactation. The increase in peripheral IGF-Ⅰ together with higher preprandial concentrations of glucose and insulin and lower NEFA concentrations after weaning in all sows indicate changes towards an anabolic state.

Maternal protein restriction may have a result to progeny performance, and in the present study, obese piglets had a significantly lower plasma total T3 concentration than lean piglets, which was in agreement with observations in obese mice (York et al., 1978). We suspect that the intensive manipulation limited the protein intake for all experimental sows and caused decreased milk production compared with sows well-managed. As a result, T_3 concentrations was reduced on d 0, and 15 during lactation, but T_4 concentrations only changed on d 35 of lactation. Because about 40% of T_4 is metabolized to T_3 through peripheral metabolism and T_3 has two to three times greater biological potency than T_4 (Ingbar and Woeber, 1974), T_3, instead of T_4, has been considered as the thyroid hormones is their effect on calorigenesis.

Previous studies have demonstrated that maternal nutrition restriction in swine during the period of maximal placental growth restricts individual placentome growth and results in a smaller placenta at mid-gestation, but has no effect on fetal weight at either mid or late gestation. In order to reduce fetal growth in late gestation it appears necessary to severely restrict maternal nutrition. Fetal weight was unaffected when sows were fed 50% of protein requirements during gestation. Furthermore, maternal undernutrition in late gestation results in enhanced neuripeptide Y mRNA abundance in the fetal hypothalamus near to term, again in the absence of any effect on fetal body or fetal

weight. Taken together, all of these findings indicate that maternal undernutrition may alter tissue function in the fetus.

These changes are likely related to the metabolic and/or endocrine changes that occurred in sows. Further research is needed to elucidate the underlying physiological mechanism.

3. IMPLICATIONS

Low protein intake during gestation and lactation seems to increase piglets body protein mobilization, and dietary protein restriction of sows seems to decreased piglets visceral organ mass ,and had a negative effects on plasma hormones levels of piglets, it indicative of the preservation of a function of the conceptus. As a result of dietary protein restriction during lactation, proportions of visceral organ decrease in weaning piglets in lactation. The comparison of dietary restriction vs. maintenance feeding MeiShan sows resulted in a similar total fetal organ mass developing within each sow type. Additional stresses placed on maternal metabolism to provide inadequate substrates for optimal fetal growth and subsequent postnatal growth of piglets may result in similar responses throughout the splanchnic tissues of the maternal system.

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Acknowledgement:

This work was supported by the National Key Basic Research Program（Grant No. 2004CB117505）

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Table 3 The effect of maternal underntrition on visceral organ mass of the newborn piglets*

Treatment	Heart [g]	Spleen [g]	Liver [g]	Kidney [g]
Control (d0 of lactation)	6.55±0.85	1.11±0.14^{A}	27.35±4.18^{A}	3.66±0.48^{A}
Treatment (d0 of lactation)	5.77±1.15	0.93±0.12^{B}	20.63±2.37^{B}	3.05±0.75^{B}
Control (d35 of lactation)	34.94±6.76	10.99±0.76^{A}	174.03±16.39^{A}	20.48±2.41^{A}
Treatment (d35 of lactation)	28.88±6.72	13.10±1.44^{B}	145.3±20.34^{B}	14.37±2.04^{B}

$^{A-B}$means within a column with no common superscript differ significantly ($p<0.01$) .

*Data represents the mean value for each treatment (Mean±SD, n=8).

Means in the same array with the different superscript small letters differ significantly（$P<0.05$）, Means in the same row with the different superscript capital letters differ significantly（$P<0.01$）, without letter superscript mean no significant difference（$P>0.05$）.

Table 4 The effect of maternal underntrition on visceral organ mass of the newborn piglets, when scaled to EBW*

Treatment	Heart [%]	Spleen [%]	Liver [%]	Kidney [%]
Control (d0 of lactation)	0.70±0.08	0.12±0.01	2.92±0.35^{A}	0.39±0.04
Treatment (d0 of lactation)	0.67±0.05	0.12±0.02	2.56±0.36^{B}	0.37±0.03
Control (d35 of lactation)	0.52±0.08	0.17±0.02^{A}	2.49±0.19^{A}	0.29±0.03
Treatment (d35 of lactation)	0.51±0.08	0.23±0.03^{B}	2.62±0.43^{B}	0.26±0.05

$^{A-B}$means within a column with no common superscript differ significantly ($p<0.01$) .

*Data represents the mean value for each treatment (Mean±SD, n=8).

The ratio of organ weight to EBW=g/kg.

Means in the same array with the different superscript small letters differ significantly（$P<0.05$）, Means in the same row with the different superscript capital letters differ significantly（$P<0.01$）, without letter superscript mean no significant difference（$P>0.05$）.

Table 5 The effect of maternal underntrition on hormone concentrations of the newborn piglets*

Treatment	T3 [ng/ml]	T4 [ng/ml]	GH [ng/ml]	INS [μIU/ml]	IGF-Ⅰ [ng/ml]
Control(d0 of lactation)	1.22±0.28^{A}	31.43±5.30	1.38±0.34^{A}	5.90±0.95	174.81±24.75
Treatment (d0 of lactation)	0.83±0.16^{B}	39.39±7.87	0.81±0.13^{B}	5.76±0.75	163.99±24.53
Control(d15 of lactation)	2.26±0.80^{A}	37.69±2.10	2.03±0.90^{A}	12.29±5.12	78.02±13.93
Treatment (d15 of lactation)	1.41±0.19^{B}	32.34±6.34	0.79±0.11^{B}	8.56±2.22	86.72±2.66
Control(d35 of lactation)	2.02±0.63	53.87±2.29^{A}	2.68±0.75^{A}	2.84±1.29	83.92±2.10
Treatment (d35 of lactation)	2.04±0.59	27.45±5.01^{B}	0.75±0.19^{B}	2.33±0.88	77.94±13.84

$^{A-B}$means within a column with no common superscript differ significantly ($p<0.01$) .

*Data represents the mean value for each treatment (Mean±SD, n=8).

Means in the same array with the different superscript small letters differ significantly（P<0.05）, Means in the same row with the different superscript capital letters differ significantly（P<0.01）, without letter superscript mean no significant difference（P>0.05）.

Figure 1. Plasma profiles of GH concentrations in piglets suckling the sows fed a low-(L=50%CP, n=8) or control (C=100%CP, n=8) protein diet during lactation.

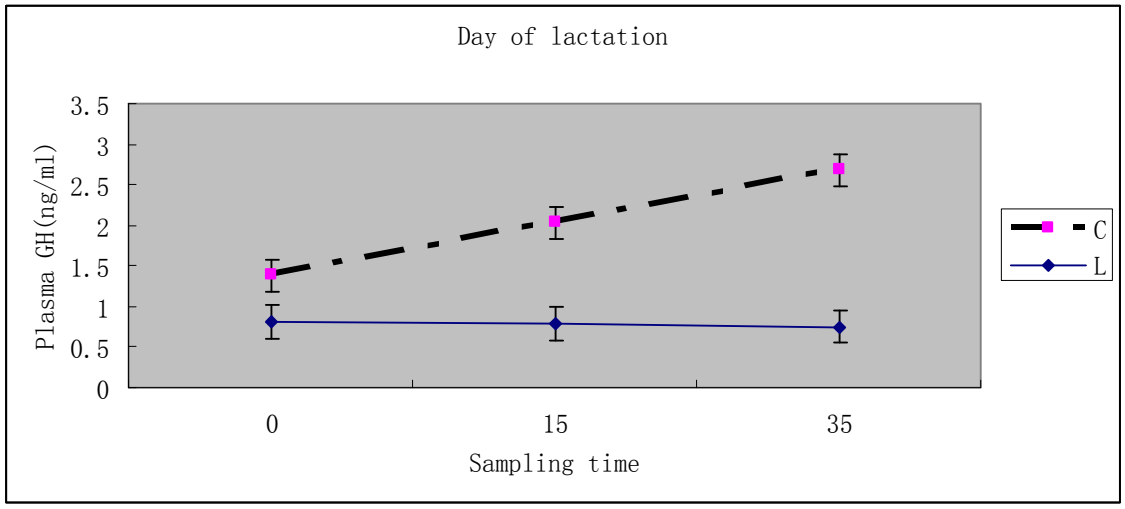
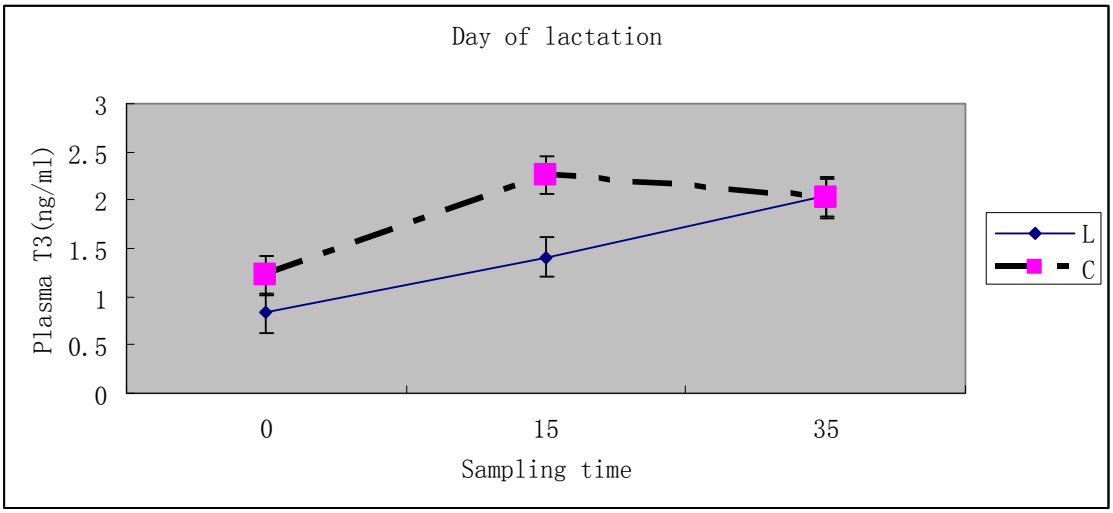


Figure 2. Plasma profiles of T_3 concentrations in piglets suckling the sows fed a low-(L=50%CP, n=8) or control (C=100%CP, n=8) protein diet during lactation.



6/12/2009