Expression of Serum and Muscle Endocrine Factors at Antemortem and Postmortem Periods and Their Relationship with Pig Carcass Grade

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ABSTRACT : Carcass weight and backfat thickness are primary yield grading factors. Insulin-like growth factor (IGF)-I/-II, transforming growth factor β1 (TGF-β1), and epidermal growth factor (EGF) regulate the proliferation and differentiation of cells including adipocytes. Also, interleukin (IL)-2/-6, cortisol, and dehydroepiandrosterone-sulfate (DHEA-S) are known to be related to muscle growth and fat depth. However, the relationships between endocrine factors and carcass grade have not been studied. Therefore, this study aimed to measure the concentrations of endocrine factors in serum and muscle, and to investigate the relationship of endocrine factors with carcass grade. A total of 60 crossbred gilts (Duroc×Yorkshire×Landrace) were used. Blood from the jugular vein was collected at antemortem (7 days before slaughter) and postmortem periods, and *M. Longissimus* was collected at 45 min and 24 h after slaughter. The concentrations of IGF-I/-II, EGF, TGF-β1, IL-2/-6, cortisol and DHEA-S were analyzed by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). In general, IGF and EGF concentrations in serum and muscle of grade A carcasses were found to be higher than those of grade C carcasses at antemortem and postmortem periods, whereas the pattern of TGF-β1 concentration was reversed. In particular, the concentrations of muscle IGF-I (24 h postmortem) and serum TGF-β1 (antemortem) were significantly different between grades A and C ($p<0.05$). The present results indicate that serum and muscle growth factors affect carcass weight and backfat thickness, and indirectly suggest the possibility that carcass grade could be predicted by expression of serum and/or muscle growth factors. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 716-722)***

Key Words : Endocrine Factor, Growth Factor, Carcass Grade, Pig

INTRODUCTION

Carcass weight, backfat thickness, and meat quality are preferentially used in the determination of carcass grades. In particular, carcass weight and backfat thickness have also been of use for various aspects of breeding and feeding management (Corino et al., 1999; O'Quinn et al., 2000; Szabo et al., 2001). Consequently, several genetic and nutritional studies have been conducted with the goal of improving carcass weight and backfat thickness (Sutton et al., 1997; te Pas et al., 1999; McNamara and Pettigrew, 2002). However, few related endocrinological studies have been reported.

Endocrine factors, such as growth factors, cytokines and hormones, are reportedly related to animal metabolism and physiology (Underwood et al., 1986; Brun, 2002). In particular, several growth factors are multifunctional proteins that regulate proliferation and differentiation of cells via autocrine, paracrine and endocrine mechanisms (Rotwein, 1991; Jones and Clemmons, 1995; Marques et al., 2000). Moreover, there have been many reports that growth factors regulate muscle development and lipid metabolism (Bass et al., 1999; Cowherd et al., 1999; Berk, 2001). Insulin-like growth factors (IGFs) -I/-II are 7.6 and 7.5 kDa polypeptides composed of 70 and 67 amino acids, respectively. Generally, IGF-I mediates the actions of growth hormones (Rotwein, 1991) and modulates proliferation and differentiation of various types of cells (Jones and Clemmons, 1995). In muscle cells, IGF-I increases myofiber diameter and number (Bass et al., 1999; Florini et al., 1996), and regulates myogenin expression (Sarbassov et al., 1995). The expression of serum IGFs is also correlated with the development of adipose tissue (Lamberson et al., 1996; Clarke et al., 1997; Marques et al., 2000). Other growth factors are also known to be involved in muscle development and adipose growth. Epidermal growth factor (EGF), a 6 kDa acidic polypeptide, has strong mitogenic activity for various cell types. EGF stimulates proliferation and differentiation of adipocytes (Butterwith, 1997). Also, there is evidence that EGF plays a role in skeletal muscle growth in growing pigs (Peng et al., 1997). Transforming growth factor (TGF)-β1 is a disulfide-linked homodimer protein that regulates proliferation and differentiation depending on cell types. In particular, TGF-β1 inhibits proliferation and differentiation of adipocytes (Ignotz and Massadue, 1985; Sparks et al., 1992; Rahimi et al., 1998) and myofibroblasts (Khouw et al., 1999), and suppresses adipogenesis (Choy and Derynck, 2003). These findings suggest the possibility of regulation of carcass weight and backfat thickness by growth factors.

There are several studies showing the involvement of hormones in muscle development and adipose growth (Goodman, 1994; Ebisu et al., 1995; Sterin-Borda et al.,

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Table 1. Korean grading system of pork carcasses (Korean Ministry of Agriculture Notification, 2001-38)

Primary carcass grade	Carcass weight (kg)	Backfat thickness (mm)
A	76 - 90	15 - 25
B	70 - 75	13 - 28
	76 - 90	13 - 24
	76 - 90	26 - 28
	91 – 93	13 - 28
C	64 - 69	11 - 31
	70 - 93	11 - 12
	70 - 93	29 - 31
	94 - 96	11 - 31
D	Not belonging to A, B, or C	Not belonging to A, B, or C

1996; Kunesova et al., 2002). On such study demonstrated the influence of dehydroepiandrosterone-sulfate (DHEA-S) on the proliferation and differentiation of adipocytes (Lea-Currie et al., 1998). In addition, a recent study by Yoon et al. (2001) suggested that hormones are related to carcass traits in pigs.

Collectively, the findings of all these reports make strong implications that endocrine factors affect carcass weight and backfat thickness. Therefore, the present study was performed to measure the concentrations of endocrine factors in serum and muscle at antemortem and postmortem periods, and to investigate the relationship between endocrine factor expression and carcass grades.

MATERIALS AND METHODS

Animals

A total of sixty 21-week-old crossbred gilts (Duroc× Yorkshire×Landrace) were used. Pigs were slaughtered by electrical-stunning, and exsanguinated for 5 min, and then scalded at 65°C hot water. After evisceration, carcasses were weighed and backfat thickness was also measured at the 11th and last thoracic vertebrae. The carcasses were graded according to the Korean Grading System for Animal Products (Table 1) and placed in the chill room.

Samples

Blood was collected from the jugular vein at antemortem (7 days before slaughter) and postmortem (45 min and 24 h) periods. The *Longissimus* muscle was collected from the right side of each carcass and stored at -80°C. Sera were made by allowing blood samples to stand at room temperature for 2 h, and then subjecting them to centrifugation at 1,000×g for 30 min. Aliquots were stored at -70°C until use.

Protein extraction

The *Longissimus* muscle was mixed with pre-chilled lysis solution (0.1 M sodium phosphate, 10 mM EDTA, 10 mM EGTA, 10 mM sodium fluoride, 1% sodium deoxycholate, 1% Triton-X100, 0.1% sodium dodecylsulfate, 1 mM phenylmethylsulfonyl fluoride, 200 kallikrein of aprotinin/ml, pH 7.2) (Burr et al., 1980). The mixture was chopped, incubated for 30 min at 4°C, and centrifuged at 10,000×g for 20 min at 4°C. The supernatant was stored at -70°C until use.

IGF radioimmunoassay (RIA)

The recombinant human IGFs-I/-II (GroPep, Pty Ltd., North Adelaide, Australia) were iodinated by the chloramine-T method (Lee and Henricks, 1990). Iodinated IGFs-I/-II were purified on a Sephadex G-50 column and aliquots of labeled tracer were stored at -20°C until use.

IGFs-I/-II in serum and muscle were analyzed by the procedure of our previous report (Yun et al., 2001) using anti-human IGF-I/-II polyclonal antiserum (GroPep. Pty Ltd.).

TGF-β1, EGF, IL-2 and IL-6 ELISA

Concentration of TGF-β1 was determined by TGF-β1 DuoSet ELISA Development System (R & D System Inc., Mineapolis, Minesota, USA). To measure the active form of TGF-β1, samples were diluted with Dulbecco's phosphate-buffered saline. Then, 1 μl of 1 N HCl was added to 50 μl of diluted sample, and the mixture was incubated for 15 min at room temperature (22-25°C). After incubation, 1 μl of 1 N NaOH was added and the neutralized mixture was used for the assay. Flat-bottom 96 well plates were coated with TGF-β1 coat monoclonal antibody (mAb) and the captured TGF-β1 was bound by a specific second polyclonal antibody (pAb). The amount of specifically bound pAb was detected using a species-specific antibody conjugated to horseradish peroxidase.

Concentrations of EGF and IL-2/-6 were analyzed by DuoSet ELISA Development System (R & D System Inc.) with minor modifications (Yoon et al., 2001).

Cortisol and DHEA-S RIA

Concentrations of serum cortisol and DHEA-S were analyzed using a Coat-A-Count Cortisol and DHEA-S kit (Diagnostic Products Co., Los Angeles, California, USA) according to standard procedure. This analysis is based upon competition between labeled antigens and non-labeled antigens for binding to a limited number of specific sites on the antiserum coated tubes. After the incubation, the liquid in the tubes was removed by aspiration and the radioactivity was measured in a gamma counter (5002 Cobra System; Packard Inst. Co., Meriden, Connecticut, USA).

Statistical analysis

Data were analyzed by analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, North

Table 2. Carcass weight and backfat thickness in gilts for different carcass grades

Carcass grade	Carcass weight (kg)				Backfat thickness (mm)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
A (n = 25)	85.56[a]	3.97	77	90	18.56[a]	2.64	15	23
B (n = 19)	87.89[a]	5.02	75	93	15.95[b]	3.34	13	23
C (n = 9)	78.14[b]	4.18	69	82	13.67[b]	4.21	11	24
D (n = 7)	91.11	19.03	66	109	14.00	4.15	10	20

[a,b] Mean values with different superscripts within the same column are significantly different ($p<0.01$).

Table 3. Concentrations of serum growth factors by carcass grades in gilts[1]

Carcass grade	IGF-I (ng/ml)		IGF-II (ng/ml)		EGF (pg/ml)		TGF-β1 (ng/ml)	
	Antemortem	Postmortem	Antemortem	Postmortem	Antemortem	Postmortem	Antemortem	Postmortem
A	254.94	262.81	320.08	301.42	104.38	73.40	30.96[a]	35.02
(n = 25)	(26.84)	(21.11)	(22.09)	(33.73)	(10.92)	(10.73)	(1.52)	(1.99)
B	240.70	243.48	306.12	286.58	87.28	81.74	34.38[ab]	40.12
(n = 19)	(34.88)	(24.76)	(32.38)	(39.26)	(12.66)	(9.23)	(1.12)	(2.73)
C	206.74	236.20	256.14	273.58	84.14	65.80	38.33[b]	43.03
(n = 9)	(37.45)	(34.71)	(26.60)	(37.90)	(7.41)	(9.35)	(2.61)	(4.38)

[a,b] Mean values with different superscripts within the same column are significantly different ($p<0.05$).
[1] All values are expressed as mean and standard error (SE).

Table 4. Concentrations of muscle growth factors by carcass grades in gilts[1]

Carcass grade	IGF-I (ng/mg)		IGF-II (ng/mg)		EGF (pg/mg)		TGF-β1 (ng/mg)	
	45 min*	24 h**	45 min	24 h	45 min	24 h	45 min	24 h
A	8.03	7.41[a]	13.18	10.63	123.96	115.43	3.45	3.71
(n = 25)	(0.81)	(0.50)	(2.63)	(2.90)	(17.20)	(15.61)	(0.46)	(0.70)
B	6.97	6.69[ab]	14.83	10.73	134.59	75.75	4.47	4.07
(n = 19)	(0.56)	(0.58)	(5.20)	(4.02)	(17.86)	(16.66)	(0.75)	(0.38)
C	6.66	5.27[b]	8.44	7.78	90.65	91.10	5.13	4.84
(n = 9)	(1.13)	(0.55)	(2.63)	(2.81)	(4.82)	(13.62)	(1.37)	(0.90)

* 45 minutes postmortem, ** 24 h postmortem.
[a,b] Mean values with different superscripts within the same column are significantly different ($p<0.05$).
[1] All values are expressed as mean and standard error (SE).

Carolina, USA) for a completely randomized design. When the main effect was significant ($p<0.05$), least squares means separation was accomplished by the PDIFF option (a pair-wise *t*-test).

RESULTS

Carcass weight and backfat thickness

Table 2 shows carcass weight and backfat thickness of pigs by carcass grade. Generally, carcass weight and backfat thickness of grades A, B and C ranged from 69 to 95 kg and from 11 to 23 mm, respectively. However, the values of grade D did not fit within those ranges, showing higher variations. Therefore, data obtained from pigs in grade D were not considered for statistical analysis.

IGF-I/-II, TGF-β1 and EGF levels in serum and muscle

To investigate the relationship between growth factors and carcass grade, the levels of growth factors were analyzed in serum and muscle at antemortem and postmortem periods.

Serum concentrations of IGF-I in grade A did not have significant differences with those of other grades, but grade A generally showed higher concentrations of serum IGF-I than grade C at antemortem and postmortem periods (Table 3). Muscle concentrations of IGF-I in grade A did not have significant differences with those of other grades at 45 min postmortem, although the lowest concentration was observed in grade C. However, a significant difference between grades A and C was found at 24 h postmortem ($p<0.05$, Table 4). On the other hand, in general, IGF-II concentrations of serum and muscle in grade C were the lowest among all the grades at antemortem and postmortem periods (Tables 3 and 4). In addition, similar patterns of EGF expression were observed. In contrast, serum and muscle TGF-β1 concentrations of grade A were lower than those of grade C at antemortem and postmortem periods. Most notably, serum levels of TGF-β1 in grade A had a significant difference with that of grade C at antemortem ($p<0.05$, Table 3).

Cytokine and hormone levels in serum and muscle

To investigate the possible effect of cytokines and hormones on carcass grade, the concentrations of cytokines and hormones were measured and compared on the basis of carcass grade. Serum concentration of IL-2 in grade B was

Table 5. Concentrations of serum and muscle cytokines by carcass grades in gilts[1]

Carcass grade	Serum				Muscle			
	IL-2 (pg/ml)		IL-6 (pg/ml)		IL-2 (pg/mg)		IL-6 (pg/mg)	
	Antemortem	Postmortem	Antemortem	Postmortem	45 min*	24 h**	45 min	24 h
A	102.80	133.70	66.78	88.92	27.04	24.85	51.85	22.61
(n = 25)	(6.25)	(6.61)	(7.28)	(7.03)	(5.67)	(3.35)	(6.32)	(3.05)
B	115.41	147.11	69.97	94.27	24.44	29.15	47.60	29.29
(n = 19)	(10.49)	(7.77)	(6.45)	(10.05)	(4.34)	(3.70)	(5.45)	(4.56)
C	105.76	148.47	67.98	85.16	18.87	26.16	53.46	26.92
(n = 9)	(12.18)	(12.50)	(6.30)	(11.82)	(3.84)	(3.57)	(13.20)	(6.77)

* 45 minutes postmortem. * 24 h postmortem.
[1] All values are expressed as mean and standard error (SE)

Table 6. Concentrations of hormones by carcass grades in gilts[1]

Carcass grade	Cortisol (ng/ml)		DHEA-S (ng/ml)	
	Antemortem	Postmortem	Antemortem	Postmortem
A	40.62	152.99	8.35	23.81
(n = 25)	(3.15)	(13.07)	(1.94)	(2.32)
B	48.77	157.04	11.29	25.07
(n = 19)	(4.67)	(16.55)	(1.600	(2.08)
C	42.02	157.55	9.22	23.34
(n = 9)	(6.54)	(26.52)	(2.70)	(4.09)

[1] All values are expressed as mean and standard error (SE).

higher than that in other grades at antemortem and that of grade C at postmortem. although these difference were not significant (Table 5). Similarly. the grade B concentrations of serum IL-6 were generally higher than those of other grades at antemortem and postmortem periods; again these differences were not significant (Table 5). In muscle. the lowest IL-2 levels was observed in grade C at 45 min postmortem, whereas grade A concentration was found to be the lowest at 24 h postmortem (Table 5). IL-6 concentration of grade B in muscle was lower than that of other grades at 45 min postmortem, and concentration of grade A was the lowest at 24 h postmortem. although these difference were not significant (Table 5). Cortisol concentrations of grade A were the lowest and DHEA-S concentrations of grade B were the highest among all grades at both antemortem and postmortem periods (Table 6). but no significant differences were not detected.

DISCUSSION

Endocrine factors are important regulators for animal metabolism and physiology (Underwood et al., 1986; Brun. 2002). Since carcass grade of pigs is mainly determined by carcass weight and backfat thickness. the reports that several growth factors regulate on the myogenesis (Sarbassov et al., 1995) and adipogenesis (Marques et al., 2000) imply that endocrine factors may affect carcass grade. In particular, IGF-I and TGF-β1 play important roles in processes such as proliferation and differentiation of muscle and fat cells (Pampusch et al., 1990; Bony et al., 1994; Marques et al., 2000; Choy and Derynck. 2003; Pampusch et al., 2003; Yun et al., 2003b). For these reasons. we measured concentrations of endocrine factors in serum and muscle in order to investigate the relationship between endocrine factors and carcass grade.

The serum levels of IGFs in grades A and C did not show any significant differences. however. the muscle concentrations of IGF-I in grades A and C were significantly different ($p<0.05$. Table 4). In general, IGFs-I/-II interact with IGF receptors and IGF binding proteins (IGFBPs), which stimulate DNA synthesis. cell proliferation. protein synthesis and glucose transport (Underwood et al., 1986; Jones and Clemmons. 1995). The report that IGF-I treatment significantly increased the growth rate and fat accretion in pigs (Schoknecht et al., 1997) was supported by the finding that IGF-I expression in serum and muscle had a positive correlation with the body growth and muscle mass (Barton-Davis et al., 1998). Moreover. serum concentration of IGF-II in pigs genetically selected for high backfat thickness was higher than that in pigs selected for low backfat thickness (Buonomo and Klindt. 1993). These reports indicate that IGF-I and -II are involved in muscle development and lipogenesis. suggesting that regulate carcass weight and backfat thickness in pigs. A positive correlation between serum concentrations of IGF-I/-II and carcass weight and backfat thickness in gilts has already been demonstrated (Yun et al., 2003a). The present study also showed that grade A carcasses expressed both higher backfat thickness and higher serum and muscle IGF-I/-II levels than grade C carcasses. These findings suggest that the expression of serum and muscle IGF-I/-II directly affect carcass weight and backfat thickness in pigs.

Adachi et al. (1995) have observed that subcutaneous fat tissues and adipocyte weight significantly decreased during anti-EGF treatment. Also. it has been suggested that the elevation of EGF might play a role in the induction of obesity (Kurachi et al., 1993). These studies point toward a positive relationship between EGF expression and fat deposition. Although the concentrations of serum and muscle EGF between grades A and C showed no significant differences. it is of interest that grade C. with lower backfat thickness. showed a tendency toward lower EGF expression.

TGF-β1 inhibits the proliferation of myogenic and smooth muscle cells (Pampusch et al., 1990; Ma et al., 2000) and reduces preadipocyte differentiation (Richardson et al., 1992), indicating the inhibitory roles of TGF-β1 in muscle development and fat deposition. In accordance with these findings, pigs with low backfat thickness in grade C showed higher serum and muscle TGF-β1 levels than pigs with high backfat thickness in grade A, during antemortem and postmortem periods. Most notably, the concentrations of serum TGF-β1 between grades A and C were significantly different at antemortem. Thus, the present study, together with previously reported finding, implies that TGF-β1 is a negative factor with respect carcass weight and backfat thickness.

It have been reported that hormones (Fraser et al., 1999; Roemmich and Rogol, 1999) and cytokines (Goodman, 1994; Ebisu et al., 1995; Sterin-Borda et al., 1996) are related to fat deposition and muscle growth. In particular, cortisol plays a role in muscle growth via IGF-I (Dwyer and Stickland, 1992) and DHEA-S may have an anti-obesity effect related to subcutaneous fat depth (Clore, 1995; Wise et al., 1995). However, the present study did not show these types of significant difference and showed that cortisol and DHEA-S varied in different ways with carcass grade, indicating that hormones and cytokines may not have direct affects on grade. Otherwise, hormones and cytokines have various functions relating to animal physiology. Cortisol and DHEA-S are mainly related to stress (Shaw and Trout 1995; Ryu et al., 1996), and act as a physiological response against stress (Parker et al., 1985; Boudarene et al., 2002). Also, cytokines such as IL-2 and IL-6, which are regulating factors in immune systems, have been related to stress (Abraham, 1991; Kanaoka et al., 2002). Thus, the results of Table 5 and 6 indicate that carcass traits such as carcass weight or backfat thickness may be influenced by environmental factors including stress, without causing any variation in carcass grades.

In summary, the present study investigated the relationship of serum and muscle levels of endocrine factors with carcass grade in pigs. At antemortem and postmortem periods, the grade A carcasses generally showed higher levels of serum and muscle IGF-I/-II, as well as EGF, than grade C carcasses. In contrast, serum and muscle TGF-β1 concentrations of grade A were found to be lower than those of grade C. Moreover, the concentrations of muscle IGF-I and serum TGF-β1 between grades A and C showed significant differences, as did carcass weight and backfat thickness between these grades. Therefore, we suggest that serum and muscle growth factors may influence carcass weight and backfat thickness, and indirectly suggests that concentrations of growth factors may be used to determine carcass grade in pigs.

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