

Plasma Insulin, Metabolite Concentrations, and Carcass Characteristics of Japanese Black, Japanese Brown, and Holstein Steers¹

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ABSTRACT: To characterize some of the physiological features of Japanese beef breeds, plasma concentrations of insulin and metabolites and carcass composition were measured in five Japanese Black, five Japanese Brown, and four Holstein steers (6.2 mo; 164 kg). The steers were raised under typical feeding conditions in Japan until they were slaughtered at 600 to 700 kg BW. Blood samples were collected at 8 mo of age (average BW, 194 kg) and at 300, 400, 500, and 600 kg BW. Plasma insulin concentrations increased with BW in all three breeds and were greater ($P < .05$) in Japanese Blacks than in the Japanese Browns or Holsteins at 400 and 600 kg BW. The Japanese Blacks exhibited lower ($P < .05$) plasma glucose levels at 300, 400, and 600 kg BW compared with Holsteins. Regardless of the breed, plasma urea nitrogen (PUN)

concentrations increased with BW. The two Japanese breeds had greater ($P < .05$) PUN levels than Holsteins at 300 and 600 kg BW. Total cholesterol and phospholipid concentrations tended to decrease above 300 kg BW in the Holsteins; however, the concentrations of both metabolites were elevated in the steers of Japanese breeds at 500 and 600 kg BW ($P < .05$). Breed did not affect the plasma concentrations of albumin, triglycerides, and NEFA. The Japanese breeds had higher ($P < .01$) dressing percentage, greater ($P < .05$) carcass fat proportion, and a lower proportion of carcass bone ($P < .01$) than the Holsteins. These results indicate that there are breed differences in plasma levels of insulin and certain metabolites and carcass composition among Japanese breeds and Holstein.

Key Words: Insulin, Blood Composition, Carcass Composition, Japanese Cattle Breeds, Beef Cattle

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Introduction

Beef breeds such as Japanese Black and Japanese Brown, which are known as Wagyu, have a unique fat deposition pattern characterized by a greater extent of marbling (Lunt et al., 1993). Ozutsumi et al. (1984) reported that Japanese breeds had greater carcass fat proportions and lesser carcass lean and bone proportions than Holsteins. The endocrine system regulates fat deposition in meat animals. In particular, insulin plays an important role in lipid metabolism, stimulating lipogenesis and inhibiting lipolysis. Blood insulin

concentration increases with age and BW in cattle (Trenkle, 1970; Martin et al., 1979). Several workers (Roy et al., 1983; Grigsby and Trenkle, 1986; Beeby et al., 1988) have reported greater insulin concentrations in smaller, early-maturing breeds of cattle than in larger, late-maturing breeds, although the converse has also been noted (Verde and Trenkle, 1987). Trenkle and Topel (1978) reported that plasma insulin concentration was positively correlated with carcass fat and negatively correlated with carcass muscle. Blood metabolite concentrations fluctuate with nutritional status, and attempts have been made to use these fluctuations as indicators of the adequacy of feeding management (Blowey et al., 1973; Russel and Wright, 1983). Differences in the energy requirements for gain and maintenance in different maturity-type steers (Chestnutt et al., 1975) indicate that the partitioning of nutrients to various body tissues differs among breeds. Thus, breed of cattle probably affects blood metabolite concentrations. Because no data on endocrine and metabolic profiles of Wagyu breeds are available, the present study was conducted to characterize genotypic differences in plasma concentrations of insulin and metabolites and in carcass traits among

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Table 1. Composition of concentrate diets (as-fed basis)

Item	Growing diet ^a	Finishing diet ^b
Ingredient, %		
Corn	35.0	40.0
Barley	35.0	40.0
Wheat bran	12.0	10.0
Soybean meal	10.0	7.9
Fish meal	5.0	—
Sodium chloride	.7	1.0
Calcium phosphate	2.2	1.0
Mineral premix ^c	.10	—
Vitamin premix ^d	.15	.10
Calculated nutrient values ^e		
CP, %	16.6	13.0
TDN, %	73.0	74.0

^aThis was used in the first period from the start of the experiment until the steers attained 400 kg BW.

^bThis was used in the second period from when the steers attained 400 kg BW to the time of slaughter.

^cEach kilogram of mineral premix contained: Mn, 50 g; Fe, 50 g; Cu, 10 g; Zn, 60 g; I, 1 g.

^dEach kilogram of vitamin premix contained: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; α -tocopherol, 10 mg.

^eCalculated from data in the Standard Tables of Feed Composition in Japan (Agriculture, Forestry, and Fisheries Research Council Secretariat, 1987).

Japanese Black, Japanese Brown, and Holstein steers when they were fed under conditions that are typical for Japan.

Materials and Methods

Five Japanese Black, five Japanese Brown, and four Holstein steers, approximately 6 mo of age, were individually housed in tie stalls. Steers were fed a concentrate (16.6% CP and 73.0% TDN) at a rate of 1% of BW fresh matter per day with Italian ryegrass hay and(or) corn silage freely available until body weight was 400 kg (first period). The amount of concentrate was adjusted every 14 d. After the steers had attained 400 kg BW, they had free access to another concentrate (13.0% CP and 74.0% TDN) and Italian ryegrass hay until the time of slaughter (second period). They were turned out together on a clay paddock from 0900 until 1100. Water and trace mineralized salt were available freely. Body weight was measured at the beginning of the experiment, at 14-d intervals, and at the end of the experiment. Feed intake was recorded in the first and second parts of the experiment. The composition of the concentrate diets is shown in Table 1.

The steers were slaughtered at a target slaughter weight of 600 kg for Japanese Black, 650 kg for Japanese Brown, and 700 kg for the Holsteins, respectively. The different target slaughter weights for the three breeds were designed to reduce the influence of physiological maturity among breeds for the com-

parison of carcass characteristics (Okada et al., 1975; McClelland et al., 1976). Steers were slaughtered after a 48-h period of feed deprivation and 24 h of water intake restriction. After an overnight chill at 0°C, the left half of each carcass was dissected into bone, lean, fat, and the other tissues including tendon and fascia. Muscle samples were obtained from the 9th to 10th rib portion of the longissimus muscle to determine the amount of moisture, crude protein, and extractable lipid in the totally trimmed muscle. Moisture content was determined by oven-drying a wet sample at 105°C for 16 h (AOAC, 1990). Crude protein was determined with the Kjeldahl method using potassium sulfate and copper sulfate as catalyst (AOAC, 1990). Lipid was extracted with diethyl ether for 16 h using a conventional Soxhlet apparatus (AOAC, 1990).

At 8 mo of age (average BW across three breeds, 194 kg), and at 300, 400, 500, and 600 kg BW, blood samples were taken at 0800, 1200, and 1600 via an indwelling catheter previously inserted into the jugular vein. Sodium heparin was used as an anticoagulant. The blood sample was immediately chilled in a cooled container (0 to 7°C) and plasma was harvested by centrifugation at $1,710 \times g$ at 4°C for 15 min. The plasma was stored at -30°C until required for analysis.

The plasma insulin concentration of all samples collected was assayed with a radioimmunoassay using an anti-bovine insulin guinea pig serum (Code No. 270400, Seikagaku-Kogyo, Tokyo, Japan), according to the method of Sasaki and Takahashi (1980). Bovine insulin (catalog number 890-8125IG, 28.2 IU/mg, GIBCO BRL, Gaithersburg, MD) was used as the reference standard, and [¹²⁵I]human insulin (code number IM166, Amersham, Little Chalfont, U.K.) was used as the tracer. All samples were assayed in a single assay and intraassay coefficient of variation was 3.2% (n = 12).

The plasma concentrations of the following metabolites were determined from the samples collected at 0800 at each sampling stage. Commercial kits (Wako Pure Chemicals, Osaka, Japan) were used for the analysis of glucose with the glucose oxidase-peroxidase method (glucose B-test), urea nitrogen with the diacetylmonoxime method (urea nitrogen-test), albumin with the bromocresol green method (albumin B-test), triglycerides with the acetylacetone method (triglyceride-test), total cholesterol with the cholesterol oxidase-phenol method (cholesterol C-test), phospholipids with the permanganate digestion method (phospholipid-test), and NEFA with the acyl CoA synthetase-acyl CoA oxidase method (NEFA C-test), respectively.

The mean insulin concentration of three samples taken at 0800, 1200, and 1600 was calculated as representative for each sampling stage. Effects of breed on plasma insulin and metabolite concentrations were analyzed using the GLM procedure with

Table 2. Performance data of Japanese Black, Japanese Brown, and Holstein steers^a

Item	Japanese Black	Japanese Brown	Holstein
No. of steers	5	5	4
Initial wt, kg	138 ± 8 ^c	195 ± 13 ^d	157 ± 11 ^{cd}
Final wt, kg	623 ± 6 ^g	642 ± 21 ^{gh}	689 ± 20 ^h
Slaughter age, mo	24.6 ± .5 ^g	25.7 ± 1.7 ^{gh}	28.6 ± .6 ^h
ADG, kg/d ^b	.87 ± .03	.81 ± .08	.77 ± .04
DM intake, kg/d ^b	6.00 ± .12 ^e	6.20 ± .19 ^{ef}	6.69 ± .19 ^f

^aValues are means ± SEM.

^bMeans for the whole experimental period.

^{c,d}Means with different superscripts differ ($P < .01$).

^{e,h}Means with different superscripts differ ($P < .05$).

^{g,h}Means with different superscripts differ ($P < .10$).

repeated measures (SAS, 1988). Because there was no consistent relationship observed for any of the plasma measurements and dry matter (DM) intake across the breeds or sampling stages, these variables were not adjusted for DM intake. Indeed, the analysis of plasma measurements for each stage was computed using DM intake for the corresponding experimental period as a covariate and revealed that the effect of this covariate on the significance of breed effect was negligible. Effects of breed on performance and carcass measurements were analyzed using GLM procedures with one-way analysis of variance. When breed effect or the breed × time interaction was significant, Duncan's multiple range test was used as a means separation test. Values are expressed as means ± SEM. Simple correlation coefficients were calculated between average plasma insulin concentration of all sampling stages and carcass composition in each breed.

Results

Performance data for the Japanese Black, Japanese Brown, and Holstein steers are presented in Table 2. The actual average ages of the cattle at the start of the experiment were 6.1, 6.9, and 5.6 mo for Japanese Black, Japanese Brown, and Holstein, respectively. Initial weight of the Japanese Browns was heavier ($P < .01$) than that of Japanese Blacks, and Holsteins were intermediate. As intended, the Holsteins tended to have a heavier ($P < .10$) final weight than the Japanese Blacks. Average daily gain for the whole experimental period was not different among the three breeds. At slaughter, the Holsteins tended to be older ($P < .10$) than the Japanese Blacks, reflecting the heavier final weight and similar growth rates. Roughage percentages in DM consumed were not different among three breeds and were 54% in the first period, 21% for the second period, and 35% for the whole experimental period. Average DM intake during the whole experimental period for the Holsteins was

Table 3. Slaughter and carcass data of Japanese Black, Japanese Brown, and Holstein steers^a

Item	Japanese Black	Japanese Brown	Holstein
No. of steers	5	5	4
Slaughter wt, kg	595 ± 6 ^e	611 ± 17 ^{ef}	655 ± 17 ^f
Dressing percentage, % ^b	64.4 ± .2 ^c	65.4 ± .2 ^c	60.2 ± .9 ^d
Carcass composition, %			
Bone	11.5 ± .2 ^c	12.3 ± .4 ^c	15.3 ± .4 ^d
Lean	54.4 ± .8	54.4 ± .7	52.5 ± .5
Fat	30.0 ± .8 ^e	29.3 ± .9 ^e	26.1 ± .4 ^f
Other tissues	4.2 ± .3 ^c	4.1 ± .4 ^c	6.2 ± .4 ^d
Chemical composition of longissimus muscle, %			
Moisture	67.3 ± .9	69.5 ± 2.2	70.0 ± .8
Crude protein	19.7 ± .3	20.8 ± .9	21.7 ± .3
Extractable fat	10.9 ± 1.3	8.6 ± 2.8	7.3 ± 1.0

^aValues are means ± SEM.

^bCalculated as hot carcass weight/slaughter weight.

^{c,d}Means with different superscripts differ ($P < .01$).

^{e,f}Means with different superscripts differ ($P < .05$).

greater ($P < .05$) than that for the Japanese Black steers.

Data on carcass measurements of the three breeds of steers are presented in Table 3. The Japanese breeds had greater ($P < .01$) dressing percentages than the Holsteins. The proportions of bone and the other tissues in the left half of the carcass were greater ($P < .01$) in Holsteins than in the Japanese breeds. Conversely, carcass fat proportion was greater ($P < .05$) in Japanese breeds than in the Holsteins. Extractable lipid content in longissimus muscle tended to be higher in the Japanese Blacks than in the Holsteins, but the difference was not significant ($P = .45$) because of a large amount of variation. Moisture and crude protein content of the muscle did not differ among the three breeds of steers.

Plasma insulin concentrations in the steers during the experimental period are shown in Figure 1. The 8 mo of age data are shown at the average BW of all steers. Plasma insulin concentrations increased with increasing BW from 3.3 to 124.3 $\mu\text{U}/\text{mL}$, from 10.8 to 45.2 $\mu\text{U}/\text{mL}$, and from 2.6 to 73.9 $\mu\text{U}/\text{mL}$, respectively, for the Japanese Black, Japanese Brown, and Holstein breeds. The Japanese Black steers had greater ($P < .05$) plasma insulin levels at 400 kg BW than the other two breeds and at 600 kg BW than the Japanese Browns.

Plasma metabolite concentrations in the three breeds of steers during the experimental period are shown in Figure 2. The 8 mo of age data are shown at the average BW of all steers. Glucose concentrations in Holsteins were consistently greater than in the Japanese Blacks, and the values for the Japanese Browns were intermediate. The differences between the Japanese Black and Holstein cattle were significant ($P < .05$) at 300, 400, and 600 kg BW. Urea nitrogen concentrations increased with BW in all

breeds of steers. The Holsteins had lower ($P < .05$) urea nitrogen concentrations than the Japanese breeds at 300 and 600 kg BW. Plasma concentrations of total cholesterol and phospholipids in the steers showed similar trends and were similar among breeds for the first period. Total cholesterol and phospholipid concentrations tended to decrease above 300 kg BW in the Holsteins; however, the concentrations of both metabolites were elevated in the Japanese Blacks at 500 kg BW ($P < .05$) compared to the other breeds. At 600 kg BW, total cholesterol concentrations were greater ($P < .05$) in the Japanese breeds than in the Holstein cattle, whereas phospholipid concentrations were higher ($P < .05$) only in Japanese Browns compared with the Holsteins. There was no difference in plasma concentrations of albumin, triglycerides, and NEFA among the breeds.

The correlations of plasma insulin level with carcass composition by breed are shown in Table 4. The plasma insulin was not significantly positively related to carcass fat proportion common to all breeds (P -values were .12, .06, and .18 for Japanese Black, Japanese Brown, and Holsteins, respectively). There was no consistent relationship between plasma insulin and carcass bone and lean proportion among the three breeds.

Discussion

Generally, Holstein cattle are considered to have greater growth potential than Japanese breeds. In the present experiment, however, Holstein steers tended to show slightly lower ADG than the Japanese Blacks. The slower growth rate of the Holsteins can be attributed to the relatively lower initial weight of animals for steers of this genotype. Also, the restricted amount of concentrate fed in the first period was probably insufficient for the expression of the higher growth potential of Holsteins. The higher ADG of the Japanese Black steers may have been caused by

Table 4. Correlation coefficients of plasma insulin level with carcass composition by breed

Item	Insulin, $\mu\text{U/mL}^a$		
	Japanese Black	Japanese Brown	Holstein
No. of observations	5	5	4
Carcass composition ^b			
Bone	-.24	-.38	.71
Lean	-.82	-.71	-.56
Fat	.78	.86	.82

^aAverage plasma insulin concentration of each steer for the whole experimental period.

^bWeight percentages of bone, lean, and fat physically dissected from carcass.

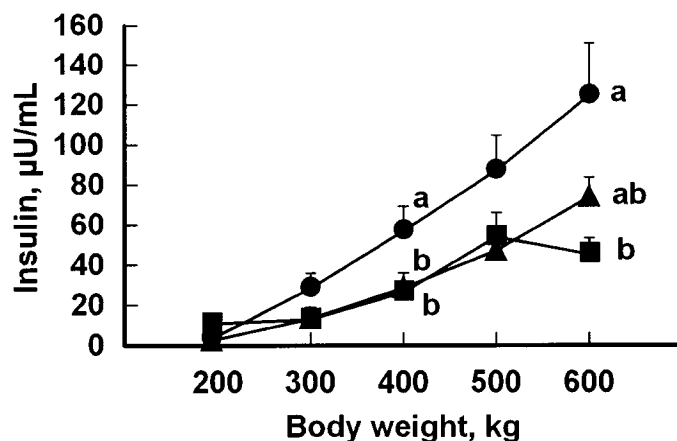


Figure 1. Plasma insulin concentrations in three breeds of steers during growing and fattening periods. Data for three samplings (0800, 1200, and 1600) were averaged to obtain a single insulin mean for each steer at each respective sampling stage. Points are means of values from five Japanese Black (●), five Japanese Brown (■), and four Holstein (▲) steers. The data points at 8 mo of age are depicted against an average BW of all steers. Standard errors are indicated by vertical bars extending from data points. Different letters (a, b) indicate significant ($P < .05$) breed effects within each sampling stage.

compensatory growth, because their initial weight and pre-experimental ADG ($.60 \pm .04$ kg/d) were less than the average for this breed. The differences in DM intake seem to be a reflection of the different target slaughter weights. The Holsteins, which were scheduled to have the heaviest slaughter weights, would have required more feed to gain and maintain their weight during the late part of the second period.

Despite the greater slaughter weight, the Holsteins had smaller carcasses as a proportion of live weight and lower proportion of carcass fat than the two Japanese breeds. Indeed, beef steers deposited more fat and were energetically more efficient when fed above a maintenance level than dairy steers (Garrett, 1971). Ozutsumi et al. (1984) compared the carcass composition of culled cows and found that Japanese Blacks and Japanese Browns had lower proportions of carcass bone and lean and a greater proportion of carcass fat than Holsteins. Present results agree well with those data. Thus, the Japanese breeds seem to have a greater propensity for fat deposition into carcass than the dairy breed. Lunt et al. (1993) clearly showed that American Wagyu steers, which were produced by crossbreeding Japanese Black, Japanese Brown, Angus, and Hereford \times Angus, had a higher ability to deposit marbling fat than purebred Angus steers. However, the present study did not demonstrate the high marbling ability of Japanese breeds. Mitsumoto et al. (1989) reported that the

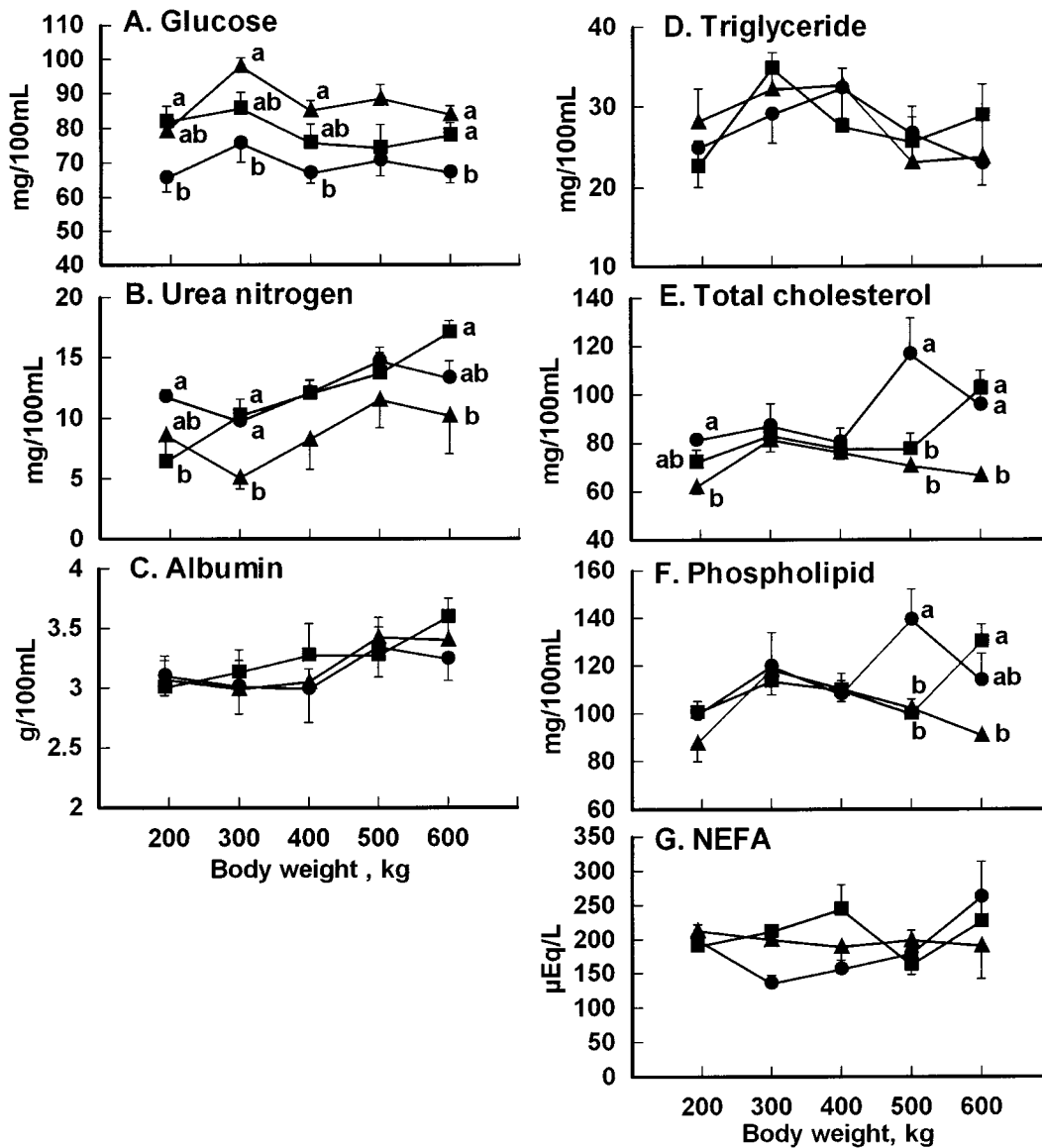


Figure 2. Plasma concentrations of glucose (A), urea nitrogen (B), albumin (C), triglycerides (D), total cholesterol (E), phospholipids (F), and nonesterified fatty acids (NEFA) (G), in three breeds of steers during growing and fattening periods. Points are means of values from five Japanese Black (●), five Japanese Brown (■), and four Holstein (▲) steers. The data points at 8 mo of age are depicted against an average BW of all steers. Standard errors are indicated by vertical bars extending from data points. Different letters (a, b) indicate significant ($P < .05$) breed effects within each sampling stage.

degree of marbling in Japanese Black steers is affected by sire of steers and(or) the major feedstuff in the diet. In the present study, all steers were fed on the same diet and were treated similarly. Hence, the difference in the degree of marbling could be attributable to the genotype of the steers. Nevertheless, the lipid contents in longissimus muscle did not differ among the three breeds of steers. All of the Japanese Black steers used in this study were sired by one Japanese Black bull. The sire of the Japanese Blacks might not have been a superior sire of this breed with respect to marbling. Limited genetic variation of the Japanese Black steers may be in part responsible for

the lack of difference in marbling among the three breeds in this study.

Regardless of breed, plasma insulin levels increased with BW in this study. This corresponds with the increase in plasma insulin with increasing age and time on feed reported in bulls (Martin et al., 1979) and steers (Trenkle, 1970; Trenkle and Topel, 1978; Ozawa, 1991). McCann and Reimers (1986) reported that obesity in cattle was associated with basal hyperinsulinemia, insulin resistance, and a greater insulin response to glucose. Sato et al. (1984) also reported that greater insulin secretion and insulin insensitivity were associated with the progress of

fattening in cows. The elevated plasma insulin levels with increasing BW noted in this study would be a compensation for the insulin insensitivity associated with fatness. Plasma insulin levels were highest in small early-maturing Japanese Blacks, but plasma glucose concentrations were lowest in these steers. This relationship between plasma levels of insulin and glucose is in good agreement with the accepted role for insulin in the regulation of glucose metabolism. This breed difference in plasma insulin is similar to the result reported by Grigsby and Trenkle (1986), who observed higher plasma insulin concentrations in small-framed Angus steers than in larger Limousin or Simmentals. Other workers (Roy et al., 1983; Beeby et al., 1988) have also reported higher levels of insulin in Aberdeen Angus-cross animals than in other late-maturing steers. Conversely, Verde and Trenkle (1987) reported that large steers had higher insulin concentrations than small steers, which was related to the greater feed intake of the large animals. In our study, the Japanese Black steers had the lowest DM intake. Therefore, the differences in plasma insulin levels between breeds in this study are probably caused by a genetically determined difference rather than the result of the differences in feed intake of the different breeds of steers.

Plasma insulin concentration was positively related to carcass fatness in cattle (Trenkle and Topel, 1978) and pigs (Wood et al., 1977). In the present study, a positive relationship between plasma insulin concentration and carcass fat proportion was observed in all three breeds, although this was not significant because of the small numbers of animals used. Furthermore, the Japanese Black steers, which had the highest levels of plasma insulin, had a higher carcass fat proportion than the Holsteins. However, there was no difference in carcass fatness between the Japanese Blacks and Japanese Browns, although the plasma insulin levels were greater in the Japanese Blacks than in the Japanese Browns. Also, carcass fatness did differ between the Japanese Brown and Holstein breeds despite the fact that plasma insulin levels were similar in the two breeds. Gregory et al. (1982) measured insulin secretory response to an intravenous tolbutamide injection in Hereford and Holstein steers. The insulin response was greater at an older age in both breeds, and it was lower in the fatter Hereford breed. They concluded that a higher insulin secreting ability is not necessarily a cause or result of fatness in ruminants. As mentioned above, the carcass composition of the Japanese Blacks and the Japanese Browns was similar, but the plasma insulin level was different between the two Japanese breeds. This raises the possibility that the effectiveness of insulin in the regulation of lipid metabolism includes β -cell sensitivity to insulinogenic signals; adipocyte responsiveness or sensitivity to the hormone is different among the breeds exhibiting similar degree of carcass fatness. It

is therefore likely that the genetic background of cattle should be taken into account when examining the relationship between fatness and plasma insulin levels. Because insulin is not the only determinant for carcass fatness, involvement of other hormones such as growth hormone or insulin-like growth factors in determining the carcass composition of Japanese breeds remains to be established.

Plasma glucose and urea nitrogen are considered to reflect the amount of ingested starch and protein or the ratio of these nutrients consumed (Blowey et al., 1973). Although the relationship between plasma glucose levels and DM intake in this study agree with this implication, the extent of differences in plasma glucose between the Japanese Blacks and Holsteins was considerably large. Thus, the observed breed differences may be due in part to a distinct breed difference in glucose turnover attributable to the rate of hepatic glucose output (gluconeogenesis) and peripheral glucose utilization. In addition, the plasma urea nitrogen levels were lower only in Holsteins, which ate more feed. The exact reasons and mechanisms for this breed difference in plasma urea levels is not known; presumably genetic differences among the breeds, which were originally bred for different purposes, may influence the partitioning of nutrients into various body tissues. A gradual increase of urea nitrogen with increasing BW seems to reflect the surplus of substrate for protein metabolism as the process of fattening progresses.

Elevation of plasma phospholipids and total cholesterol levels in the Japanese breeds were observed in the late fattening period. This agrees with previous results indicating that plasma phospholipid and cholesterol concentrations increased with increasing age of steers from 9 to 16 mo (Brungardt et al., 1963; Brungardt and Bray, 1966). Because no elevation of plasma phospholipid and total cholesterol was observed in the leaner Holsteins, carcass fatness seems to be associated with the elevated plasma levels of phospholipid and cholesterol.

In summary, the present results showed that there were intrinsic breed differences in plasma levels of insulin and certain metabolites, the Japanese Black having remarkably greater plasma insulin than the other breeds. Carcass measurements provide evidence that Japanese breeds have proportionally more fat and less bone in the carcass than Holsteins when the steers are fed under similar conditions. These differences are probably attributable to the heredity of the cattle, which were originally bred for different purposes.

Implications

The present results provide an initial indication that Japanese Black steers have greater plasma

insulin levels during the fattening period than Japanese Brown and Holstein steers. Breed differences in certain blood metabolite concentrations were also observed among Japanese beef breeds and Holsteins during the growing and fattening period. Japanese breeds seem to have a greater propensity to deposit carcass fat than Holsteins when the steers are raised under the typical feeding conditions used in Japan. Research is needed to clarify the relationship between the endocrine system and carcass characteristics, especially with respect to marbling.

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