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Effects of Glucocorticoid on Components of Blood Serum and Amino Acids Composition of the Serum

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Summary

For induction of fat deposit in meat, hypothyroidism is the most important factor. This study was carried out to confirm the effect of glucocorticoid on thyroidal function, using rats.

Triamcinolone acetonid, a synthetic compound was used as glucocorticoid. Rats of an experimental group were injected singly subcutaneously with 0.9 mg of the compound and another group of rats served as control. The animals were sacrificed by decapitating at 2 hours and 4 hours following the injection. At the time of decapitating, blood was obtained from the animal respectively.

A half portion of the blood serum was used for determination of such blood components as total protein (TP), albumin (Alb), globulin (Gl), blood ureal nitrogen (BUN), glucose (Glu), cholesterol (Chol), calcium (Ca), magnesium (Mg) and phosphorus (P). Another half portion of the serum was subjected to amino acid analysis, using a automatic amino acid analyzer.

By injection of the glucocorticoid, concentrations of TP, Alb, and Gl decreased, and concentration of amino acids increased. This is due to gluconeogenesis induced by glucocorticoid. Serum phosphorus was elevated, and ratio of Ca/P decreased and P/Mg increased. These are indicative of lowered thyroidal function. Accordingly, glucocorticoid seemed to have a favourable effect on fattening cattle.

Results of amino acid assay were summarized in Table 2. Most of amino acids increased by the injection of the compound, except glutamic acid and taurine. This is due to protein catabolism and gluconeogenesis. Concentration of serum glucose consistently increased. Increase of glycine concentration in serum is indicative of hyperthyroidism. This is, however, due to neoglucogenesis by cortisol. Low concentration of Mg is indicative of hyperthyroidism.

Further study for the effect of glucocorticoid would be required, since glucocorticoid is considered to accelerate production of lean meat.

Introduction

Adrenal cortical steroids influence carbohydrate and mineral metabolism. Cortical steroids concerning carbohydrate metabolism are called glucocorticoid and cortical steroids concerning mineral metabolism are called mineral corticoids.

Adrenal cortical steroids influence protein metabolism as well. These hormones in relatively high concentration cause a dissolution of lymphoid tissue, a decrease in total leucocyte count, a decrease in the absolute number of polymorphonuclear cells. Simultaneously there is a rise in the total serum protein which appears to be principally in the globulin fractions.

In the adrenalectomized animals, such symptoms as hemoconcentration, decreased blood pressure and blood flow, loss of appetite, poor gastrointestinal absorption and diarrhea were observed. These effects seem to give a great influence on the nutrition of animal.

The adrenal glucocorticoids received their name from their function to cause deamination of

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amino acid. Following the removal of the NH_2 group, the carbohydrate residue of many amino acids can be converted to glucose in the liver. As a result, the blood glucose can increase. The deamination, however, decreases the availability of amino acids for somatic growth or the synthesis of milk protein and thus depresses growth and milk secretion.

Glucocorticoid has an important role in production of carbohydrate, namely, glycogen and finally glucose from protein[9]. The carbohydrate turnover is called gluconeogenesis or neoglycogenesis. The process also seemed to be important in fattening cattle, particularly in improvement of quality of beef. An important role of corticoid in milk production has been proved by many workers[10]. However, a few data are available in the field of physiology of meat production and actual beef production[3,13]. This study was carried out to clarify the physiological effect of glucocorticoid in meat production, using rats.

Materials and Methods

Sixteen male rats of the Wister strain, weighing about 130g were used in this study. They were divided into three groups consisting of 8, 4 and 4 rats respectively. The first group of 8 rats was served as control. The other groups consisting of 4 rats were injected with synthetic glucocorticoid. The synthetic glucocorticoid used was triamcinolone acetonide and its aqueous suspension, containing 6 mg of the compound per ml, was injected in a dose of 0.15 ml equivalent to 0.9 mg of triamcinolone acetonide. The rats of the second group were sacrificed by decapitating at 2 hours following the injection, and the rats of the third group at 4 hours.

The blood was taken individually at the time of decapitating, kept in the room temperature for 10 hours, and then centrifuged to separate the serum. The rats of the control group were sacrificed without injection and their blood were taken by the same way.

Blood sera of 5 rats in the control group were subjected to determination of blood components, and blood sera of 3 rats were subjected to amino acid analysis. Blood sera of rats of the second group and the third group were divided into 2 portions respectively, and one portion of each serum was subjected to determination of blood components and another portion of each serum was subjected to amino acid analysis.

Blood components examined for their serum concentration were total protein (TP), albumin (Alb), globulin (Gl), glucose (Glu), blood ureal nitrogen (BUN), cholesterol (Chol), Ca, Mg, and P. The components were determined, using Vet-Aid, a automatical degital type spectrophotometer using electronics, made by Fujihira Industrial Co., Ltd. The portion of blood serum examined for their amino acids composition was subjected to procedure of amino acid analysis after elimination of protein by the method previously described[14].

Results and Discussion

1. Effect of glucocorticoid on blood serum components.

Results of determination of concentration of the blood component were shown in Table 1. Concentration of total protein, albumin, globulin decreased, while concentration of glucose increased by the injection of glucocorticoid. This is due to gluconeogenesis induced by the effect of the glucocorticoid. Decreased concentration was observed in blood ureal nitrogen as well. This is thought to be indication of increased neoglucogenesis. Serum concentration of cholesterol decreased markedly by the injection. This shows that glucocorticoid has an effect on lipid metabolism.

Serum concentration of calcium and magnesium decreased, while concentration of serum phosphorus increased. Therefore, ratios of Ca/P and Mg/P decreased. This seems to be a hypothyroidal state[15] and glucocorticoid seems to decrease the function of thyroid gland[12].

Glucocorticoid stimulates the formation of phosqholipids in the liver. Increase of the phosphorus may be related to mobilization of phosphorus for the formation of phospholipids. Serum concentration of total protein, albumin, and globulin decreased. This must be due to catabolism

Table 1 Serum concentration of blood components in rats treated with glucocorticoid

	Control	Glucocorticoid 0.15 mg, 2 hrs.	Glucocorticoid 0.15 mg, 4 hrs.
Initial b.w., g	126±9	146±11	128±13
Final b.w., g	143±23	180±12	151±28
No. of rats	5	4	4
Period, days	7	7	7
TP, g/dl	3.88±1.70	2.20±0.21	2.66±0.36
Alb, 〃	2.90±1.39	1.25±0.16	2.08±0.24
Gl, 〃	0.90±0.31	0.95±0.05	0.58±0.12
Glu, mg/dl	81.60±27.75	92.00±21.73	98.75±10.40
BUN, 〃	14.28±3.92	9.90±2.84	10.68±1.97
Chol, 〃	129.40±53.96	20.00±8.68**	23.75±12.42**
Ca, 〃	9.16±1.04	6.33±0.95**	6.38±1.17*
Mg, 〃	3.26±0.78	1.88±0.10*	1.98±0.22*
P, 〃	7.46±0.99	9.68±1.13*	8.73±1.65
Alb/Gl	3.2 ±0.4	1.3 ±0.3	3.6 ±0.5
Ca/P	1.24±0.15	0.68±0.05**	0.73±0.05**
Ca/Mg	2.91±0.59	3.40±0.63	3.28±0.83
P/Mg	2.35±0.35	5.18±0.76**	4.48±1.10*

* $P < 0.05$, significant difference to control.
** $P < 0.01$, significant difference to control.

of protein in neoglycogenesis.

Stimulative effect of glucocorticoid on thyroidal function in cattle depends on kind and dose of the glucocorticoid injected. Relation of adrenal corticoid to function of thyroid is somewhat complicated. Glucocorticoid stimulates in one case, and depresses in another case. The individual differences in response to glucocorticoid in cattle is not known, but may be related to individual secretion rate of other hormones[11] and to dose of glucocorticoid.

2. Effect of glucocorticoid on concentration of amino acid in blood serum.

The result of amino acid analysis was shown in Table 2. Concentrations of serum taurine and glutamic acid decreased respectively by the injection of glucocorticoid. Serum concentration of other amino acids increased by the injection. Particularly, concentration of serine, glutamine, valine, isoleucine, leucine, phenylalanine and histidine increased more than 50% of their control levels.

Concentration of threonine, methionine, phenylalanine, and ornithine was elevated by the injection of the hormone. Concentration of phenylalanine almost doubled its control level. The concentration of the four amino acids was apparently higher at 4 hours than 2 hours following the injection. The increase in serum concentration of amino acid is a result of protein catabolism. This indicated that amounts of amino acids mobilized from the tissue were more than those absorbed by the liver for neoglucogenesis.

Concentration of asparagine, proline, glycine, and lysine in serum was elevated by the injection and they remained stationary at 2 hours and 4 hours following the injection. Their increases in serum concentration due to the injection of glucocorticoid were rather moderate in contrast to the marked increases of phenylalanine, valine, histidine, serine, leucine, and so on. Their increased concentrations were 70% higher than their control levels.

Concentration of serum glutamine, alanine, isoleucine, leucine, tyrosine, histidine and arginine was elevated by the injection of glucocorticoid. The concentration of these amino acids was lower at 4 hours than at 2 hours following the injection. Decreased concentration of amino acids seemed to be due to uptake of amino acids by the liver for gluconeogenesis.

The thyroid state of rat has caused changes in the plasma[8]. Most of plasma amino acids are

Table 2 Concentration of serum free amino acid in rats treated with glucocorticoid

A.A.	Control (n = 3)	Glucocorticoid 0.15 mg ; 2 hrs. (n = 4)	Glucocorticoid 0.15 mg ; 4 hrs. (n = 4)	Ratio to control		
Tau	0.175±0.026	0.167±0.041	0.155±0.037	100	95.43	88.57
Urea	2.865±0.119	2.262±0.286*	2.838±0.639	100	78.95	99.06
Asp.A	0.043±0.016	0.058±0.010	0.050±0.017	100	134.88	116.28
Thr	0.360±0.070	0.465±0.100	0.509±0.062*	100	129.17	141.39
Ser	0.206±0.031	0.321±0.056*	0.356±0.042**	100	155.83	172.82
Asp-n	0.054±0.005	0.062±0.022	0.059±0.012	100	114.81	109.26
Glu.A	0.083±0.007	0.082±0.009	0.071±0.004	100	98.80	85.54
Glu-n	0.444±0.047	0.705±0.129*	0.661±0.132*	100	158.78	148.87
Pro	0.151±0.012	0.176±0.053	0.173±0.022	100	116.56	114.57
Gly	0.172±0.049	0.224±0.024	0.228±0.033	100	130.23	132.56
Ala	0.381±0.031	0.529±0.142	0.452±0.081	100	138.85	118.64
Val	0.094±0.002	0.162±0.033*	0.163±0.037*	100	172.34	173.40
Met	0.042±0.001	0.052±0.015	0.057±0.012	100	123.81	135.71
Ileu	0.062±0.006	0.099±0.019*	0.086±0.016	100	159.68	138.71
Leu	0.084±0.004	0.143±0.029*	0.134±0.028*	100	170.24	159.52
Tyr	0.066±0.007	0.080±0.020	0.072±0.013	100	121.21	109.09
Phe	0.032±0.003	0.058±0.012*	0.063±0.009**	100	181.25	196.88
Orn	0.048±0.007	0.056±0.013	0.070±0.016	100	116.67	145.83
Lys	0.213±0.025	0.260±0.047	0.262±0.044	100	122.07	123.00
NH_3	0.300±0.039	0.499±0.165	0.445±0.144	100	166.33	148.33
His	0.048±0.009	0.081±0.014*	0.077±0.018*	100	168.75	160.42
Arg	0.130±0.013	0.173±0.038	0.146±0.024	100	133.07	112.31
Total AA.***	2.911±0.208	3.964±0.698*	3.857±0.564*	100	136.17	132.50

* $P < 0.05$
** $P < 0.01$
*** Except Urea and NH_3.

elevated after desiccated thyroid was fed. It was reported that thyroidectomy caused a decrease in concentration of most amino acids except glycine and citrulline, concentration of which increased[8]. This fact indicates that concentrations of serum amino acids except glycine and citrulline decrease in case of hypo- or disfunction of the thyroid gland.

Amino acids are grouped into two groups, namely, antiketogenic amino acids and ketogenic amino acid[1]. The former (antiketogen) includes such amino acids as glycine, alanine, serine, cystein, cystine, hydroxyproline, arginine and histidine. These amino acids are easily converted to glucose. The latter (ketogen) includes such amino acids as leucine, isoleucine, phenylalanine, tyrosine. These amino acids are converted to keton body. In another grouping, amino acids are divided into two groups, namely, essential amino acid and non-essential amino acid. Changes in concentration of amino acids following the injection of glucocorticoid occurred without any relation to ketogenic or antiketogenic and to essential or non-essential.

It was suggested that the cells contain glucocorticoid receptors and these receptors mediate the effect of glucocorticoid on adipose tissue. These receptors contribute to fat catabolism and to regulation of plasma total lipid level[1].

Liver and mammary enzymes are not affected significantly by adrenalectomy of glucocorticoid therapy and glucocorticoid plays a less significant role in the regulation of liver and mammary enzymes and metabolism in sheep than in rats[6].

Glucocorticoid stimulates or depresses the function of thyroid gland[11] and stimulates secretion of milk[2]. Glucocorticoid increases growth rate and live weight gain in steers[7,12,13] or

increases fat synthesis or fat deposit in the muscle[2,3,4]. Thus injection of exogenous glucocorticoid is useful in production beef. However, the effect depends on kind and dose of glucocorticoid, and is not always useful.

The result obtained in this study was similar to that in the case of a hyperthyroidism in the previous report in which thyroxine was injected to the rats[5]. In other words, glucocorticoid causes protein catabolism as thyroxine does. The thyroidal gland and adrenal cortical tissue are stimulated functionally by estrogen, and their hormones are secreted in a condition of cold stress as well. Thus adrenal gland and thyroidal gland have partially a similar effect in the physhiology of meat production and in calorigenesis. The condition and environment stimulating the adrenals are not favourable to fat deposition in the muscle.

Adrenalin is the hormone secreted from the medullary tissue of the adrenal glands. It is well known that adrenalin elevates blood glucose and blood lactate and decreases glycogen content of liver and skeletal muscle. Adrenalin also raises the blood concentration of free fatty acids by increasing the activity of lipase, thus promoting hydrolysis of tryglyceride in adipose tissue to glycerol and free fatty acid. Thus, adrenalin is not favourable to fat deposition in meat. Cold temperature causes an excitement of sympathetic nerve and increases the secretion of adrenalin. Accordingly, cold stress disturbs the fat deposition in the muscle through the effects of increased hormone secreted from medullary and cortex of the adrenal glands. Conclusively, the conditions and environmental factors stimulating the adrenals are not favourable to improvement of fat deposit of beef. WADA, et al[13] demonstrated the effect of glucocorticoid on live weight gain in the steers of Japanese Black Breed of cattle. The hormone had not, however, an effect on deposition of fat in the beef in the experiment.

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ラッテの血清の成分および アミノ酸粗成に対するグルココルチコイドの効果

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肉の脂肪交雑の形成には低甲状腺機能状態が最も重要な要因である．この研究はラッテを用い甲状腺の機能に対するグルココルチコイドの効果を検討するために実施した．

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グルココルチコイドとしては合成物質である Triamcinolone acetonido を用いた．試験区のラッテに対しては此の物質 0.9 mg を 1 回，皮下注射し，残りのラッテを対照区とした．注射後 2 時間および 4 時間にラッテを犠牲にし，断頭により個々の動物の血清を採った．この血液から分離した血清の半分は総蛋白質 (TP)，アルブミン (Alb)，グロブリン (Gl)，血清尿素態窒素 (BUN)，ブドウ糖 (Glu)，コレステロール (Chol)，カルシウム (Ca)，マグネシウム (Mg)，リン (P) など血清成分の測定に用い，他の半分を自動アミノ酸分析器によるアミノ酸分析にかけた．

このグルココルチコイドの注射により TP, Alb, Gl の濃度は増加し，アミノ酸の濃度は増加した．これはグルココルチコイドにより糖新生が起きたことによるものである．血清のリン濃度が増加し Ca/P 比の減少，P/Mg 比の増加がみられた．これらは甲状腺機能の低下を示すものである．従ってグルココルチコイドは牛の肥育に対しても有益な効果をもつものと思われる．

アミノ酸分析の結果は表 2 に要約した．グルココルチコイドの注射により，グルタミン酸およびタウリン以外の多くのアミノ酸の濃度が増加した．これは蛋白質分解・糖新生によるものである．これと一致して血清の糖濃度は増加した．グリシンの濃度の増加は甲状腺機能昂進状態を示す．これは糖新生によるものである．血清 Mg の低濃度は甲状腺機能の亢進を示すものである．

グルココルチコイドは脂肪の少ない肉を生産すると考えられているので，その効果に関しては一層の研究が必要である．