

INVESTIGATIONS ON METABOLIC ALTERATIONS IN LAYING HENS FOLLOWING AMINO ACID ADMINISTRATION

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Summary: Ketogenic amino acids L-leucine and L-isoleucine were administered in amounts of 100 mg with or without 1.5 g glucose to overnight fasted laying hens. On administering leucine the blood glucose level was observed to be slightly reduced while administration of isoleucine increased the blood sugar. On injecting leucine or isoleucine with glucose blood glucose level was found to be appreciably higher as compared to that observed on administering glucose alone. *In vivo* administration of amino acids caused only a slight increase in plasma alpha-amino nitrogen. The liver glycogen was found to be reduced after the administration of amino acids. These observations indicate that amino acids leucine and isoleucine exercise a definite role on glucose uptake, and its mechanism of utilization in poultry birds is different from mammals.

Key words: L-leucine blood sugar plasma alpha-amino nitrogen
L-Isoleucine liver glycogen

INTRODUCTION

The amino acids L-leucine and L-isoleucine have a status distinct from other amino acids as regards their relation to carbohydrate metabolism. Administration of these two ketogenic amino acids have been known since long to cause hypoglycaemic response in mammals, while the administration of other amino acids results in hyperglycaemia (7, 8). The amino acids may influence glucose metabolism by affecting metabolic pathways involving glucose and also by influencing the secretion of hormones controlling these processes (4). One other distinguishing feature possessed by leucine and isoleucine, as shown by the work of Gopalan and his associates (6) is their involvement in pellagra. Excessive dietary intake of leucine may cause niacin deficiency by blocking the conversion of tryptophan to nicotinic acid. However, there is no report available to indicate whether the pellagragenic effect of leucine has any bearing on its role in carbohydrate metabolism.

MATERIALS AND METHODS

Adult poultry birds 6 in each group, including the control group, kept on deep litter system were used for the present investigations. The effect of intramuscular administration of 100 mg L-leucine, 100 mg L-isoleucine and combination of these amino acids were studied on the fasting blood sugar levels at different interval of times. Similar groups of birds were given the above amino acids along with 1.5 g of glucose to each bird intramuscularly. The glucose tolerance test was done at intervals of 10, 20, 30 and 40 minutes. These intervals of time were earlier determined to be most suitable by pilot experiments. Adequate controls were also done in each of the several groups of experiments.

Blood sugar was estimated by the method of Asatoor and King(2). Plasma alpha-amino nitrogen was measured by the method of Cocking and Yemm (3). Fresh liver tissues were used for the determination of glycogen by Phenol Sulphuric acid method as described by Montgomery (11)

RESULTS AND DISCUSSION

The blood sugar level was found to be slightly, though not significantly, reduced on administering leucine. On administering leucine with glucose this level was appreciably higher as compared to those obtained on administering glucose alone at 20, 30 and 40 min. The difference was statistically significant at 40 minute (Table I). The level of alpha-amino nitrogen in plasma was increased slightly but not significantly. Liver glycogen level was reduced significantly (Table II).

Administration of isoleucine resulted in the increase of the blood sugar level. The peak value observed at 30 min, was statistically significant as compared to the value at zero minute. On administering isoleucine with glucose the blood sugar level was found to be higher at 30 and 40 min as compared to the values obtained on injecting glucose alone. The difference at 40 min was statistically significant. The level of plasma alpha-amino nitrogen was increased, though not significantly so. The concentration of glycogen in liver was found to be reduced slightly, but not significantly.

The effects of administering leucine and isoleucine on carbohydrate metabolism in poultry birds have not been investigated so far. Some of the observations obtained in the present studies differ from those reported in man and other animals. In man, administration of leucine as well as isoleucine induce hypoglycaemia (8) whereas in the present work two amino acids showed opposite effects. Another difference observed in the

TABLE I : Effect of leucine and isoleucine with or without glucose on blood sugar level (Mean \pm S.E.).

(mg/100 ml)

Time in minutes	0	10	20	30	40
Leucine	144.5 \pm 9.04	131.87 \pm 5.4	138.83 \pm 11.07	134.83 \pm 10.13	127.71 \pm 8.0
Isoleucine	156.2 \pm 10.37	184.2 \pm 12.25	198.75 \pm 16.03	221.4 \pm 18.25*	178.75 \pm 13.9
Leucine + Isoleucine	146.77 \pm 11.93	161.52 \pm 11.01	152.2 \pm 12.38	168.81 \pm 7.74	181.3 \pm 20.36
Glucose	175.66 \pm 9.80	220.77 \pm 11.52	211.28 \pm 17.59	201.77 \pm 14.86	188.66 \pm 11.66
Glucose + Leucine	177.95 \pm 8.64	198.05 \pm 6.51	228.46 \pm 15.19	220.88 \pm 14.68	227.56 \pm 10.71*
Glucose + Isoleucine	183.80 \pm 9.55	235.30 \pm 5.42	227.40 \pm 14.91	235.30 \pm 18.19	221.90 \pm 7.45*
Glucose + Leucine + Isoleucine	140.83 \pm 7.91	147.00 \pm 6.96	176.66 \pm 5.22	177.80 \pm 7.20	173.00 \pm 8.85**

* P < 0.05.

** P < 0.01

TABLE II : Effect of leucine, isoleucine and glucose on liver glycogen and plasma alpha-amino nitrogen.

(Mean \pm S.E.)

	Liver glycogen (mg/100 g)	Plasma alpha-amino nitrogen (mg/100 ml)
Control	568.40 \pm 53.47	6.59 \pm 0.14
Glucose	678.00 \pm 33.47	5.76 \pm 0.48
Leucine	345.10 \pm 26.07*	7.17 \pm 0.30
Control	762.50 \pm 68.35	4.20 \pm 0.67
Isoleucine	704.00 \pm 22.93	4.72 \pm 0.18

*P < 0.01

present work lies in the blood sugar response to administration of a mixture of glucose and amino acid. On administering amino acid with glucose in mammals, one should expect suppression of glucagon secretion by glucose and relatively more predominant effect of insulin resulting in lowered blood sugar levels, since even normal glucose level (5mM) has been shown to suppress glucagon secretion (12). In present studies, blood sugar levels on administering leucine or isoleucine with glucose are higher as compared to administration of glucose alone; the increase being statistically significant at 40 minutes. These observations have been discussed on the basis of metabolic and hormonal control of blood sugar level.

Administration of leucine and isoleucine, or infact any amino acid may alter blood sugar level by directly influencing the metabolic pathways of glucose (13,14) and also by influencing the secretion of hormones controlling glucose metabolism (4). The hypoglycaemic effect of leucine may be partly due to the inhibitory effect of this amino acid on gluconeogenesis. Leucine has been shown to inhibit *in vitro* the incorporation of radioactive amino acids and pyruvate into glucose by the mouse liver slices (10). Leucine inhibits glycolysis as well in erythrocytes (1). Isoleucine is not known to possess any such inhibitory properties. The direct effect of amino acids on metabolic reactions are however dominated and therefore, usually masked by the effect of amino acids on hormones involved in glucose metabolism.

Although blood sugar levels alone do not give any definite indication of the amounts of insulin and glucagon secreted, some inferences can be drawn from the present work on the basis of alterations observed in blood sugar and liver glycogen levels on administering amino acids. In case of leucine and isoleucine, liver glycogen levels were found to be appreciably reduced as compared to controls. This indicates unequivocally that glucagon, and not insulin, has upper hand in case of poultry birds unlike mammals. This inference, goes very well with the informations available regarding insulin-glucagon system in poultry birds (5,9).

Glucagon plays a dominant part as evidenced by reduced liver glycogen levels, in the present series of experiments, but it does not stimulate any further insulin secretion. The hypoglycaemic response of leucine may also be due to inhibition of gluconeogenesis and glycolysis. On giving leucine and isoleucine together, blood sugar levels lie in between the levels observed in response to these amino acids administered individually. When the amino acids are administered with glucose, the blood sugar levels are higher as compared to administration of glucose alone. This indicates that utilization of glucose has not been favoured in this situation, even though exogenous glucose is likely to suppress glucagon

secretion. This observation further confirms the incompetency of insulin in utilization of glucose in poultry birds.

The present work confirms that carbohydrate metabolism in poultry birds, its regulation and relation with metabolism of amino acids is very different from mammals.

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