The Effect of Fasting on the Concentration of Free Amino Acids in the Blood Plasma of Goats

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絶食がヤギの血漿遊離アミノ酸濃度に及ぼす影響 藤 原 勉

Introduction

The investigation of fasting metabolism in goats has been reported formerly by Tasaki. Hirose and Asahida also investigated with the energy requirement for maintenance in goats using the indirect method. Recently, Fujihara et al. and Fujihara and Furuhashi reported with the heat production of goats measured by a method of tracheal cannula on the 5th, 6th and 7th days of fasting.

In sheep, the investigation in fasting metabolism has been reported by Blaxter. Leibholz and Cook reported the effect of starvation on the free amino acid in blood plasma of lambs. Leibholz also reported the effect of long periods of fasting on the nitrogen metabolism in wethers. Recently, Cross et al. reported the free amino acid concentrations in blood plasma of wethers fasted for 24 hours.

Relatively little work has been performed on the nitrogen metabolism in fasting goats, in which the changes in plasma free amino acid concentration has been discussed. The present experiment was carried out to investigate the effect of short period of fasting on the concentration of free amino acids in plasma of goats.

Experimental Procedure

Three castrated male Japanese Saanen goats, being about 2 years old and 25-28 kg in weight, were used as the experimental animal. The animals were kept in the metabolism cages throughout the experimental period. The procedure of measurement of heat production was almost similar to that reported by Fujihara and Furuhashi.

On the first, 6th and 7th days of fasting, about 5 ml of jugular blood was sampled just before the gas collection at 9 hour, and the free amino acids in plasma were determined. The plasma was deproteinized by treating with picric acid (1%) according to the procedure described by Stein and Moore, and the free amino acids were determined by ion exchange chromatography on automatic amino acid analyzer (Model KLA-5, Hitach Co, Ltd. Tokyo).

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Results and Discussion

In the present experiment, the average heat production was calculated as 1011.9 kcal per day per 50 kg body weight on the 5th, 6th and 7th days of fasting, and this result was very comparable with that reported by Tasaki (1063.9 kcal) and Fujihara and Furuh- $_{4}^{(1)}$ ashi (1036.7 kcal) with goats. Similarly, Blaxter reported with sheep that the heat production per day per 50 kg body weight was 1016 kcal in fasting condition. From the facts mentioned above, it is confirmed that the net energy requirement may be 1000-1100 kcal per day per 50 kg body weight in goats. The fecal nitrogen output of goats in the present experiment was 0.50-1.08 g/day/head on the last three days of 7-day fasting. This result was in agreement with that of Fujihara and Furuhashi (0.4-0.8 g/day), and was slightly lower than the metabolic fecal nitrogen of goats reported by Ukai (1.34 g/day) and Fujihara and Tasaki (1.74 g/day), in which the non-protein diet was introduced into the abomasum of goats. From these results, it is obvious that the goats could be in postabsorptive state at the 5-7th days of fasting.

The concentration of amino acids in plasma of goats on the first, 6th and 7th days of fasting is shown in Table 1. The concentration of most of amino acids tended to increase with the lapse of day during fasting. Of the essential amino acids, valine and threonine in the blood plasma slightly decreased with the lapse of day during fasting, and lysine,

		Days of fasting		
Amino acid	1	6	7	
Thr	$7.64 \pm 1.77*$	6.58 ± 1.07	5.92 ± 1.40	
Va1	$26.33 \pm \ 6.49$	22.19 ± 2.10	19.96 ± 4.00	
Met	$0.77 \pm \ 0.41$	$1.60\pm$ 0.33	$1.81\pm$ 0.3	
Ile	$7.76\pm$ 1.28	7.66 ± 1.44	$8.15\pm$ 1.6	
Leu	$12.90 \pm \hspace{0.15cm} 3.17$	$13.59 \pm \hspace{0.1cm} 3.31$	14.14 ± 2.50	
Phe	$5.82\pm$ 1.30	$3.71\pm$ 0.56	3.30 ± 0.74	
Lys	9.89 ± 4.29	$9.56 \pm \ 1.13$	$10.56\pm$ 1.3	
His	$7.08 \pm \ 1.43$	$4.71{\pm}0.60$	$4.20\pm$ 0.8	
Trp	Trace	$0.19\pm$ 0.19	0.84 ± 0.0	
Arg	$9.91\pm$ 1.03	$12.56\pm\ 2.99$	11.16 ± 1.4	
Asp + Asn	$5.57\pm~2.09$	$2.74\pm$ 0.90	$4.94\pm~1.49$	
Ser	14.45 ± 5.66	$5.93\pm$ 1.72	$7.64\pm~1.0$	
$Glu\pm Gln$	22.75 ± 4.08	22.82 ± 3.39	$18.03\pm$ 6.6	
G1y	73.59 ± 20.25	73.14 ± 13.02	72.59 ± 15.6	
Ala	$16.05\pm\ 3.02$	11.72 ± 2.17	$11.72\pm$ 0.8	
Tyr	$5.63\pm$ 0.27	$2.35\pm$ 0.26	$2.83\pm$ 0.3	
Orn	$6.40 \pm \ 1.20$	$7.02\pm$ 0.69	4.82 ± 2.5	
TAA	238.52 ± 26.70	207.51 ± 32.42	202.60 ± 34.5	
EAA	90.27 ± 15.63	82.40 ± 11.33	80.04 ± 11.3	
EAA/NEAA	$0.69\pm$ 0.24	$0.67\pm$ 0.05	$0.67\pm$ 0.0	

Table 1. The concentration of free amino acids in plasma of goats in fasting condition $(\mu mol/100 ml)$

* Mean±S. E. of 3 goats.

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isoleucine and leucine were not changed by fasting. Leibholz and Cook also reported the slight increase in the ceoncentarions of branched-chain amino acids and lysine in the blood plasma of starved sheep. From these results mentioned above, it is assumed that both lysine and branched-chain amino acids have a slower rate of metabolism than the other amino acids in the blood plasma. On the other hand, the concentrations of methionine and tryptophane in the blood plasma rather increased with the lapse of day during fasting. According to Schelling et al., the plasma level of methionine tended to increase with an increase of dietary nitrogen in lambs. Fujihara and Tasaki also observed that the concentration of methionine in the blood plasma was trend to increase with an increase of dietary casein level in goats sustained by abomasal feeding. These facts described above should suggest a something unique about these amino acids in the blood plasma of goats.

The concentrations of glycine, glutamic acid+glutamine and ornithine in the blood plasma were almost similar in the first, 6th and 7th days of fasting. The plasma glycine level observed in the present experiment was considerably lower than that reported by Fujihara and Tasaki, in which the non-protein diet was given into the abomasum of goats. The ^{7,8,9)} high level of plasma glycine in fasted ruminants had been reported by many workers. Cross et al. reported that the plasma glycine level was 95.3 μ m/100 ml in wethers after 24-hour fasting. This value was higher than the result obtained in the present experiment. According to Leibholz, the plasma glycine level was considerably decreased by 7-day fasting in lambs, and he suggested that the reduction of plasma glycine level may be due to the utilization of this amino acid as an energy source in the starved animals. The plasma alanine level was lower in the 6th and 7th days than that in the first day of fasting in the present experiment. This trend was very in agreement with that reported by Leibholz. Adibi also found that the starvation resulted in a decrease in the concentration of alanine in the blood plasma of man. This trend was followed in the present experiment by serine and tyrosine, and these amino acids would be also utilized as an energy source, as well as alanine and glycine.

As shown in Table 1, the total amino acid concentration in the blood plasma of goats fasted for 24 hours in the present study was very comparable with that of wethers reported by Cross et al., and the values in the 6th and 7th days during fasting were slightly higher than that of Fujihara and Furuhashi. The discrepancy between the result in this study and that obtained previously occurred despite the experimental procedure being almost the same in both experiments. This may be due to the age of experimental animals, because the concentration of free amino acid in the blood plasma decreased with aging in ruminants. The concentration of total amino acids in the blood plasma decreased in response to the lapse of day during fasting, though there was no significant difference. Similarly, the total concentration of essential amino acids in the blood plasma was lower in the 6th and 7th days of fasting than that in the first day of fasting. Consequently, the ratio of essential to non-essential amino acid concentrations was not changed with the lapse of day during fasting. According to Leibholz, the ratio of essential to non-essential amino acids in the blood plasma was 0.70 in lambs starved for 7 days, and thereafter the ratio decreased gradually. Cross et al. also reported that the ratio of essential to non-essential amino acid concentrations in the blood plasma was 0.66 in wethers fasted for 24 hours. The result

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obtained in the present experiment was very in agreement with that of Leibholz and of Cross et al.. 9^{9}

	Days of fasting		
Amino acid	1	6	7
Thr	10.4±0.9*	7.9±0.2	7.2±0.8
Va1	$28.7 {\pm} 2.2$	27.2 ± 1.1	24.5 ± 1.4
Met	$0.9{\pm}0.4$	$1.9 {\pm} 0.2$	$2.3{\pm}0.2$
Ile	$8.8{\pm}0.7$	$9.2{\pm}1.0$	10.0 ± 0.6
Leu	$14.5 {\pm} 2.0$	16.2 ± 3.0	17.5 ± 1.5
Phe	$6.4 {\pm} 0.4$	$4.7{\pm}1.0$	4.1 ± 0.4
Lys	$10.5 {\pm} 4.7$	11.8 ± 1.3	13.8 ± 3.1
His	$7.8 {\pm} 0.3$	$5.8{\pm}0.7$	5.5 ± 1.3
Arg	12.2 ± 3.2	$14.9 {\pm} 1.6$	14.0 ± 0.5
Trp		$0.3 {\pm} 0.3$	1.1 ± 0.2
Total branched-chain amino acids	$51.9{\pm}4.2$	$52.7{\pm}3.4$	52.1 ± 2.8

Table 2. Effect of fasting on proportions of essential amino acids in plasma of goats (%)

* Mean \pm S. E. of 3 goats.

Table 2 shows the changes in proportions of essential amino acids in the blood plasma due to fasting, and values are expressed as a percentage of the total essential amino acids in the plasma. The pattern of essential amino acids obtained on the first day of fasting was a little different from that obtained on the 6th and 7th days of fasting. The proportions of methionine and tryptophane tended to increase with the lapse of day during fasting. As described previously (Table 1), the concentrations of methionine and tryptophane also increased with the lapse of day during fasting. From these results, it is suggested that limiting amino acids would probably reserve in the blood plasma when the animals are under fasting condition, though there are suggestions that a limiting amino acid would 13,14) probably not accumlate in the blood plasma when the dietary protein is increased.

The proportions of branched-chain amino acids to the total essential amino acids were not changed by the 7-day fasting. This can be explained on the basis of the fact that these branched-chain amino acids are metabolized relatively slowly in the liver.

The ratios of glycine to valine and of glycine to branched-chain amino acids in the blood plasma of goats are shown in Table 3. According to Fujihara and Tasaki, the concentration of plasma glycine reduced and the proportion of valine and branched-chain amino acids increased with an increase of dietary casein level in goats sustained by abomasal feeding. Therefore, they suggested that the ratios of glycine to valine and of glycine to branchedchain amino acids in the blood plasma could serve as useful index of nutritional condition in goats. In the fasting condition, however, the concentration of plasma glycine did not change and the proportions of branched-chain amino acids also did not change with the lapse of day during fasting (Tables 1 and 2). Therefore, the ratios (glycine : valine or glycine : branched-chain amino acids) scarcely altered with the lapse of day during fasting. From these results, it is suggested that these ratios could not serve as useful index of nit-

Days of fasting	Ratio of glycine to valine	Ratio of glycine to branched-chain amino acids
1	$3.41 \pm 1.29*$	$1.87 {\pm} 0.69$
6	$3.26 {\pm} 0.31$	$1.67 {\pm} 0.06$
7	$3.62 {\pm} 0.38$	$1.70{\pm}0.13$

Table 3. Effect of fasting on amino acid ratios in plasma of goats

* Mean \pm S. E. of 3 goats.

rogen metabolism in goats when they are fasting condition.

Summary

The present experiment was carried out to investigate the effect of fasting on the concentrations of plasma free amino acids in goats, and the following results were obtained. 1. The concentration of most of amino acids in the blood plasma tended to decrease with the lapse of day during fasting, though there was no significant difference. The concentration of total amino acids in the blood plasma decreased in response to the lapse of day

during fasting.

2. Of the essential amino acids, methionine and tryptophane in the blood plasma rather tended to increase with the lapse of day during fasting. The pattern of essential amino acid obtained on the first day of fasting was little different from that obtained on the 6th and 7th days of fasting.

3. The ratios (glycine : valine or glycine : branched-chain amino acids) scarcely altered with the lapse of day during fasting. This suggestes that these ratios could not serve as useful index of nitrogen metabolism in goats when they are fasted.

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摘 要

ャギの血環遊離アミノ酸濃度に及ぼす絶食の影響について検討するため、去勢成雄ヤギ3頭を用い、連続7 日間の絶食中における血環遊離アミノ酸濃度の測定を行い、次の結果を得た。

- 血浆中遊離のほとんどのアミノ酸の濃度は絶食日数が進むにつれて有意ではないが減少する傾向があり、 同様に全アミノ酸濃度もまた減少する傾向が認められた。
- 必須アミノ酸のうち、メチオニンとトリプトファンは有意ではないが絶食日数が進むにつれて増加する傾向が認められた。全必須アミノ酸に対する個々の必須アミノの割合を示した必須アミノ酸のパターンは、絶食日数が進んでもほとんど変化しなかった。
- 蛋白質の栄養判定の一つの指標となり得るグリシンに対するバリンあるいは全側鎖アミノ酸の比率は,絶 食7日間ではほとんど変化なく,これらの比率は絶食時の窒素代謝における指標とはなり得ない事が示唆さ れた。