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PLASMA FREE AMINO ACID FRACTIONS IN DIFFERENT PHASES OF REPRODUCTION IN DWARF NANNY GOAT*

NABILA ROOHI, ABDUL MAJEED CHEEMA, NAZIA RASHID AND MUHAMMAD WAHEED AKHTAR

Department of Zoology (NR, AMC, NR) and Institute of Biochemistry and Biotechnology (MWA), University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Abstract: One dimensional thin layer chromatography was employed to detect free amino acids fractions in various phases of reproduction in dwarf goat. Cystine of band 1, observed in amino acids standard, could not be separated in samples. No appreciable variation was observed in arginine, histidine, lysine, glutamine and asparagine fractions of band 2, tryptophan, valine and methionine of band 7 and phenylalanine and 3, 4-dihydroxyphenylalanine of band 8 throughout the estrous cycle. Glycine and serine of band 3 were significantly reduced, compared to proestrus, in estrus, metestrus and diestrus phases. Threonine of band 4 and leucine and iso-leucine of band 9 were significantly enhanced in estrus compared to proestrus phase. The level was, however, restored in succeeding phases of estrous cycle. Alanine of band 5 and tyrosine of band 6 were markedly reduced in estrus compared to proestrus phase. The level was found rehabilitated in following metestrus and diestrus stages.

Arginine, histidine, lysine, glutamine and asparagine of band 2 were found to be markedly enhanced in early, mid and late gestation compared to prepregnancy estrus. Glycine and serine of band 3 were significantly depressed in mid and late gestation compared to prepregnancy estrus. Threonine of band 4 appreciably declined in early but markedly enhanced in mid and late gestation compared to prepregnancy estrus. Tyrosine of band 6 remained almost unaffected in early and mid, however, reduced in late gestation in comparison with prepregnancy estrus. Tryptophan, valine and methionine of band 7, phenylalanine and 3, 4-dihydroxyphenylalanine of band 8 and leucine and iso-leucine of band 9 remained almost in the range of prepregnancy estrus throughout the entire course of pregnancy.

Arginine, histidine, lysine, glutamine and asparagine of band 2 and threonine of band 4 were appreciably enhanced in mid and late compared to early lactation. Glycine and serine of band 3 reduced in mid and late but elevated in advanced lactational phase compared to early lactation. Alanine of band 5 and tryptophan, valine and methionine of band 7 depressed significantly in mid and late lactational phases in comparison with early lactation. Tyrosine of band 6 reduced markedly in late compared to early lactation. No significant variation was observed in phenylalanine and 3, 4-dihydroxyphenylalanine of band 8 and leucine and iso-leucine of band 9 throughout the entire course of lactation.

Key words: Dwarf goat, amino acid fractions, estrous cycle, pregnancy, lactation.

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INTRODUCTION

Amino acids are the crucial nutrients in pregnancy and lactation. Plasma amino acid pools show important variations throughout the gestational period in the rat and these are net consequences of maternal adaptations to the increased metabolic needs (Pastor-Anglada *et al.*, 1986). In spite of eventual catabolic phase during the last third of pregnancy, nitrogen retention seems to increase in pregnant rats. Furthermore, high placental transfer of amino acids maintains an adequate nutrient supply to the fetuses (Remesar *et al.*, 1987). Alanine and glycine constituted the largest proportions of all the 17 amino acids in the blood plasma in high pregnant sows (Jezkova *et al.*, 1990). In analyses, in healthy mothers after delivery, it was suggested that reduced placental gradient of amino acids could be one of the compensatory mechanisms to facilitate their adequate supply to fetus in human (Boersma *et al.*, 1980). Steingrimsdottir *et al.* (1993), as evaluated in human uterus, consider that amino acids are in excess and are not needed in anabolic processes or as a fuel.

Lactation, specifically, exerts a rapid and formidable demand on amino acids supply and skeletal muscle protein is the major source of endogenous amino acids (Mepham, 1987). Pamblanco *et al.* (1989) observed increase in amino acid fractions in earlier phase and showed decrease in the later phase of lactation.

In a monitoring of free amino acids in plasma and muscle, in dairy cow, it was noticed that compare to precalving concentrations methionine, phenylalanine, glutamine and glycine decreased by 16, 24, 25 and 25%, respectively, and the muscle protein was degraded for supply of amino acids to the udder, particularly, glycine is potentially limiting for milk protein synthesis in high yield cow. Looking into the availability of an amino acid and its consequent appearance in milk, in certain studies, raise the question to the type and source of amino acid precursors by tissue for protein synthesis (Backwell *et al.*, 1996).

Thus there is a need to follow the mechanism of metabolism of various nutrients during the entire period of reproduction. Hence, the present study was planned to observe the pattern of free amino acids fractions in plasma of dwarf goat, as a ruminant model, during various phases of its reproduction.

MATERIALS AND METHODS

Free amino acids in plasma were determined by one dimensional thin layer chromatography (Berry, 1971). Plasma free amino acids were extracted with redistilled ethanol. The extract was dried and amino acids were reconstituted in 50% isopropyl alcohol. The fractions were separated on precoated cellulose plates (5632-D. Fertigplatten cellulose, Merck). Different fractions of separated amino acids were visuallized in the form of blue violet bands while reacting with ninhydrin. Plates were immediately photographed and their images were saved on a floppy disk with image store 5000 Gel Documentation System. Amino acids in each band were identified by their Rf values. The quantification of separated amino acids fractions was carried out by

UVP gel base software programme. The system, while reading the TLC images, provides the data of the total area covered by each of the bands displayed in the form of peaks in the histogram. The data was analyzed statistically using one way analysis of variance ($P<0.05$) and employed in finding the enhancement or reduction and the appearance or disappearance of particular amino acids fractions for comparison among different reproductive phases.

RESULTS

Seventeen amino acids fractionated in eight bands, by thin layer chromatography, were detected in various phases of reproduction in dwarf goat. Cystine of band 1, observed in amino acids standard, could not be separated in samples, whereas, proline and hydroxyproline were not visible by ninhydrin reagent but they appeared as yellow spots on the plates.

Estrous cycle

No appreciable variation was observed in arginine, histidine, lysine, glutamine and asparagine fractions, throughout the different stages of estrous cycle. Glycine and serine, on the other hand, were found to be significantly reduced by 41% in estrus compared to proestrus phase. In metestrus, an elevation of 36% was exhibited by glycine and serine fractions when compared to the estrus phase, however, there was still 20% lowering in comparison with the proestrus phase. No change was exhibited by both of these fractions in diestrus compared to metestrus phase but the fractions were found to be significantly lowered by 28% when compared to the proestrus phase of the estrous cycle. Threonine was found to be significantly and pronouncedly enhanced by 128% in estrus compared to proestrus phase. The level returned in the range of proestrus in the succeeding metestrus phase, however, it was still 52% lower as compared to the estrus phase and remained in the same range even in diestrus phase. A significant decline of 24% as compared to proestrus, was exhibited by this fraction in the succeeding metestrus and diestrus stages.

Tyrosine was found to be appreciably reduced by 34% in estrus compared to proestrus phase. The level returned to the proestrus range in metestrus phase although it was found to be 56% higher as compared to estrus stage. No significant change was observable in the concentration of tyrosine fraction in diestrus phase. Tryptophan, valine and methionine fractions did not exhibit any appreciable variation throughout the entire course of estrous cycle with the exception of a 20% decline observed in metestrus compared to the estrus phase. The level of phenylalanine and 3-4, dihydroxy phenylalanine was found to be the same in all of the four stages of estrous cycle. Leucine and iso-leucine were found to be markedly elevated by 88% in estrus compared to proestrus phase. The level was found to be rehabilitated in metestrus phase, however, it was almost 43% lower as compared to the estrus phase. Pronounced elevations of 54 and 63% were observed in diestrus compared to metestrus and proestrus stages, respectively (Fig. 1 A).

Pregnancy

Concentrations of arginine, histidine, lysine, glutamine and asparagine fractions remained unaffected throughout the entire course of pregnancy.

Glycine and serine were found to be 27% lower in mid and 32% lower in late compared to early gestation. The lowerings were, however, found to be 37% and 57% respectively, in mid and late gestation when compared to prepregnancy estrus. A marked reduction of 33% was exhibited by threonine fraction in early pregnancy compared to prepregnancy estrus. Fraction was found to be pronouncedly enhanced by 177% in mid compared to early gestation. A further enhancement of 29%, compared to mid gestation, was observable in late gestation. These enhancements in mid and late gestation were even more intensified when compared to prepregnancy estrus. Alanine remained almost unaffected in early pregnancy compared to prepregnancy estrus, however, a 39% significant reduction, compared to early gestation, was observed in mid gestation. The level remained within the same range even in late gestation.

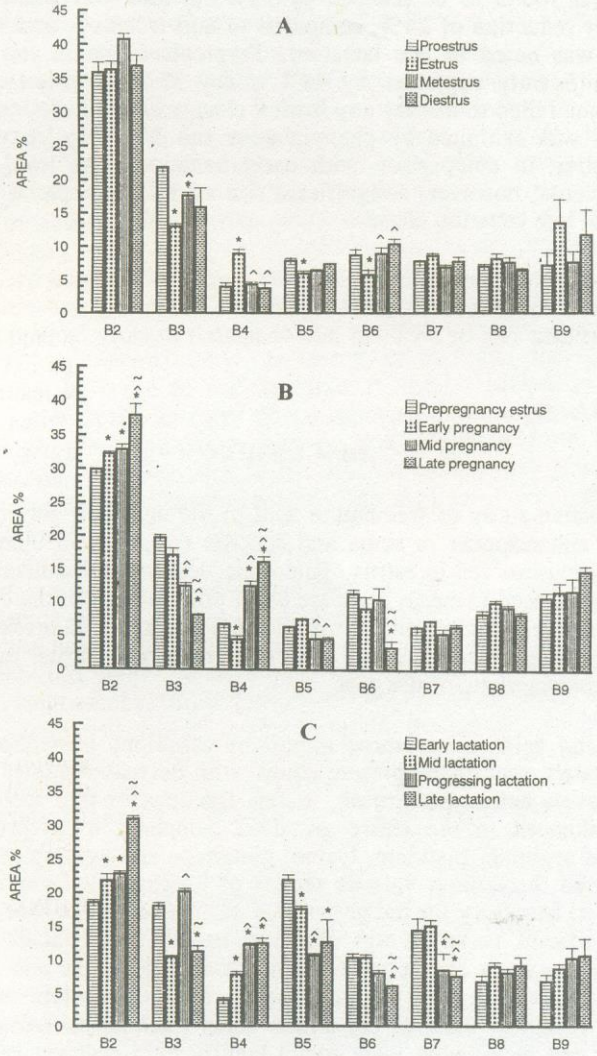
Tyrosine was found to be 20% reduced in early pregnancy compared to prepregnancy estrus. Fraction remained within the same range in mid pregnancy, however, a marked decline of 68%, compared to mid pregnancy, and 70% compared to prepregnancy estrus, was observed in late gestational phase. No significant variation was observed in tryptophan, valine and methionine fractions throughout different phases of pregnancy except in mid gestation where a significant decline of 26%, compared to early gestation, was noteworthy. Phenylalanine and 3-4, dihydroxy phenylalanine were found elevated by 20% in early gestation compared to prepregnancy estrus. The level remained almost unaffected in mid gestation and even restored to prepregnancy range in following late gestation.

Leucine and iso-leucine remained almost in the same range in prepregnancy estrus, early pregnancy and mid pregnancy, however, a 25% elevation, compared to mid pregnancy, was observed in late gestational phase (Fig.1 B).

Lactation

Arginine, histidine, lysine, glutamine and asparagine fractions remained almost unaffected throughout the entire course of lactation with the exception of 35% elevation observed in late lactation as compared to day 35 of mid lactation.

Glycine and serine were found to be highly suppressed by 42% at day 28 of mid lactation. Conversely, with advancing lactation (day 35), a highly significant elevation of 94%, compared to day 28 of lactation, was noteworthy which was found to be reduced by 45% in late lactation. Threonine was found to be highly significantly enhanced by 98% in mid (day 28) compared to early lactation. Further elevation of 58% was observed at day 35 of lactation. The level was 114% higher when compared to early lactation and remained within the same range in succeeding late lactational phase. Significant reductions of 20% and 52%, in alanine fraction, were observed at day 28 and 35 of lactation, respectively, as compared to early lactation. The fraction did not exhibit any further alteration in its concentration in late lactation and remained within the range



B2 arginine, histidine, lysine, glutamine and asparagine; B3 glycine and serine; B4 threonine; B5 alanine; B6 tyrosine; B7 tryptophan, valine and methionine; B8 phenylalanine, 3-4, dihydroxy phenylalanine; B9 leucine, iso-leucine.

Average percent areas covered by various amino acids fractions resolved into eight bands (B2-B9), in different stages of estrous cycle (A), pregnancy (B) and lactation (C) in goat. Values are mean ± SEM. *, ^ and ˜ Significances ($P < 0.05$) in relation to proestrus, estrus & metestrus (A); pre, early & mid pregnancy (B); early, mid & progressing lactation (C), respectively.

of mid lactational (day 35) value.

Tyrosine was found to be reduced by 23%, in mid (day 35) compared to early lactation. Further reduction of 24%, compared to mid lactation, and 41%, compared to early lactation, was noted in late lactation. Tryptophan, valine and methionine were found to be significantly depressed by 44% at day 35 of lactation compared to early lactation. Fractions failed to display any further change in late lactation phase. A marked increase of 34% was exhibited by phenylalanine and 3-4, dihydroxy phenylalanine at day 28 of lactation in comparison with early lactation. The level remained almost unaffected afterwards, however, a significant rise of 37%, compared to early lactation, was observable in late lactation phase.

Leucine and isoleucine were found to be markedly elevated by 28% at day 28 of lactation compared to early lactation. No further alteration was observed afterwards, however, a significant rise of 58%, in late compared to early lactation, was noteworthy (Fig.1 C).

DISCUSSION

In the extensive study of free amino acid profle and their patterns, in the cycling goat, a specific enhancement in some and specific reduction in other free amino acid fractions have been observed in estrus. Threonine, leucine and isoleucine were found to be significantly enhanced in estrus compare to all other phases of the cycle. On the other hand, glycine, serine, alanine and tyrosine were markedly depressed. The pertinent variations in the different fractions of amino acids, during estrus, may be linked with multiovulatory characteristic of the goat.

Plasma amino acids pools show important variations throughout the gestational period, in the Dwarf goat of the present study, with decreased values at mid pregnancy and recovered levels before parturition. A few fractions of free amino acids remained exceptionally enhanced in the entire gestation compared to prepregnancy estrus as concentrations of arginine, histidine, lysine, glutamine and asparagine were found to be markedly enhanced throughout various phases of pregnancy. Their increased presence may be considered necessary for the gestational adaptation by the breed to carry multiple fetuses. No significant variation was observed in free amino acids fractions in early compared to prepregnancy except for the reduction in threonine and tyrosine fractions. This indicated the involvement of these amino acids in protein anabolism of early pregnancy. The levels of most of the amino acids fractions including glycine, serine, tyrosine, valine and methionine were found significantly reduced in mid compared to early gestation. This decrease in amino acids levels, at mid pregnancy, is mainly due to the changes in gluconeogenic amino acid group. The decreased trend of maternal circulatory amino acids may also be attributed to their rapid clearance after being uptaken by the fetus. It appeared as if the availability of these amino acids through catabolism in the cireulation could not overtake the fraction's clearance by the fetus. In late gestation, glycine, serine and tyrosine fractions were markedly reduced while threonine, leucine and isoleucine were found to be appreciably enhanced. The trend of these fractions, in late pregnancy, may be attributed to the catabolic role of these in late

pregnancy. The discriminaing behaviour of free amino acid fractions has been reported by other studies. In pregnant rabbits, when hyperaminoacidemia occurs, a greater amount of gluconeogenic amino acids (glycine and serine) would escape the liver, suggesting a higher availability of these for the fetus (Pere *et al.*, 1994).

In the present study, in dwarf goat, significantly elevated concentrations of glycine, serine, alanine, tyrosine, tryptophan, valine and methionine were encountered in the entire period of lactation when compared to preparturition levels. Increased levels of these amino acids in plasma are indicator of essential amino acids availability for the lactating tissue in cow (Shmanenkov, 1986). Lopez-Tejero *et al.* (1989) also reported increased levels of plasma essential free amino acids at the peak of lactation. Moreover, the levels of arginine, histidine, lysine, glutamine, asparagine, threonine, phenylalanine, leucine and isoleucine were found to be markedly reduced in early lactation signifying their extensive uptake from plasma for the synthesis of protein rich clostrum. Several amino acids fractions including glycine, serine, alanine, tyrosine, tryptophan, valine and methionine were found significantly depressed in mid and late compared to early lactation indicating their use in milk protein synthesis. Meijer *et al.* (1985) while monitoring free amino acids, in dairy cows, reported that the plasma concentrations of methionine, phenylalanine, glutamine and glycine decreased from 6 to 15 weeks of lactation. The decrease corresponded to the order in which they generally appear to be limiting for milk protein synthesis. Probably the availability of these fractions is not sufficient for satisfactory let down of milk.

Lactation phase thus exerts heavy demand on maternal plasma metabolites for the production of milk and such situation is of greater magnitude in ruminants such as cow, sheep and goat (Baldwin and Plucinski, 1977). Lactation, specifically, exerts a rapid and formidable demand on amino acids supply and skeletal muscle protein is the major source of endogenous amino acids (Mepham, 1987). Differences in the contents and changes in free amino acids levels, during lactation, among the groups were observed by Pamblanco *et al.* (1989). Amino acids fractions increased in the earlier phase and showed decrease in the later phase of lactation. Increased levels of total plasma free amino acids are present during lactation of dwarf goat (Bogin and Lappis, 1980).

On the overall, in the present investigation, it is realized that the status of free amino acids fractions is not favourable for adequate let down of milk in this breed of goat and the metabolites setup of the goat adapted during pregnancy fails to shift to an efficient lactational level. The restoration of the levels of certain free amino acids fractions, peculiar to pregnancy, including arginine, histidine, lysine, glutamine, asparagine, threonine, phenylalanine, leucine and isoleucine, in mid lactation, soon after a short early lactation, supports this apprehension. This probably renders the lactation phase poor in performance.

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