Correlations between states of amino acids and hematology or plasma biochemistry in calves within 24 hours after birth

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Abstract

The present study examined correlations between state of free amino acids and hematological or plasma biochemistry, using blood samples acquired from Japanese-black calves within 24 hours after birth. All of free amino acids demonstrated negative correlations with red blood cell counts, hemoglobins and hematocrits. The most significant positive correlation was recorded between γ-glutamyl transpeptidase and glutamic acid, with a linear regression of y=0.025 x+39.99, where x corresponds to γ-glutamyl transpeptidase and y corresponds to glutamic acid (r=0.88, p<0.001). This suggests glutamic acid concentration may relate to the degree of colostrum intake in Japanese-black newborn calves.

Material and method

Sampling and examination

Heparinized blood samples were collected from each calf via the jugular vein within 24 hours after birth. The red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), albumin (ALB), total protein (TP), aspartate aminotransferases (AST) and GGT were measured immediately following collection. Plasma was acquired by centrifugation at 3000 x g for 10 min and stored at -80°C until measurement of free AAs. Before analysis of free AAs, a solution of 3% salicylic acid was added to samples to facilitate removal of proteins. Free AAs, urea and ammonia (NH3) were measured using an amino acid analyzer (JLC-500/V2, AminoTac, JEOL Ltd., Tokyo, Japan). Threonine (Thr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys), histidine (His), tryptophan (Trp) and arginine (Arg) were all classified as essential AAs (EAA). Aspartic acid (Asp), serine (Ser), asparagine (Asn), glutamic acid (Glu), glutamine (Gln), glycine (Gly), alanine (Ala), tyrosine (Tyr), proline (Pro), citrulline (Citr) and ornithine (Orn) were all classified as nonessential AAs (NEAA).

Statistical analysis

The Pearson correlation coefficient and p value were used to determine statistically significant relationships between the hematological or plasma biochemistry and each individual AA, EAA, NEAA, and the total AAs (TAA). SPSS statistics 17.0 was used for statistical analyses. In relation to blood states and amino acid concentrations, statistical correlations were determined at three levels of significance, p<0.05, 0.01 and 0.001.

Result

Hematological values differed greatly among individual calves; for RBC, the mean±SD was 869.2±181.1 ×10⁴ cells/µl (range, 494–1250...
×10⁶ cells/µl; Ht was 37.0±7.8% (19–51%); Hb was 11.4±2.4 g/dl (5.3–14.9 g/dl) (Table 1). Biochemical plasma values also differed greatly among individual calves, except for ALB and TP; AST was 71.0±62.0 U/l (21–352 U/l); GGT was 2362.8±2476.4 U/l (13–8753 U/l); urea was 1621.6±557.3 pmol/l (724.5–3253.3 pmol/l); ammonia was 141.2±55.6 pmol/l (29.5–248.7 pmol/l). The concentrations of free AAs in calf blood, in descending order of concentration, were Ala (270.8±140.6 nmol/ml), Gly (242.0±78.9 nmol/ml), Gln (221.9±119.5 nmol/ml), Pro (145.1±111.2 nmol/ml), and Val (138.8±57.8 nmol/ml), respectively.

Negative correlations were observed between all of the free AA concentrations and RBC (r=−0.12 to −0.74), Hb (r=−0.08 to −0.75), and Ht (r=−0.19 to −0.77) (Figure 1). With the exception of Glu, Gly, Trp, and Arg, the observed negative correlations with Ht and Hb were significant (p<0.05). Positive correlation coefficients were recorded for the free AA and ammonia, ranging from 0.10 to 0.62. Correlations were significant (p<0.05) for all AA, except Gln, Gly, Ala, Ile, Phe, Cit, and Arg. None of the free AA demonstrated significant correlation with urea (p>0.05; r=−0.20 to 0.39). The correlation coefficients between free AAs and GGT ranged from −0.50 to 0.88. Specifically, significant correlations (p<0.01) with GGT were observed for Glu (r=0.88), Trp (r=0.56), and His (r=0.54). Overall, the strongest correlation coefficients were in order as follows: the relationship between GGT and Glu, y=0.025 x+39.99, where x refers to GGT and y refers to Glu, r=0.88 (Figure 2); the relationship between Ht and TAA, y=−60.70 x+3981.20, where x refers to Ht and y refers to TAA, r=−0.77 (Figure 3); the relationship between Ht and NEAA, y=−43.00 x+2780.10, where x refers to Ht and y refers to NEAA, r=−0.76 (Figure 3).

| Blood examination Mean±SD [minimum-maximum] | Blood examination Mean±SD [minimum-maximum] | RBC (×10⁶ cells/µl) | 869.2±181.1 [494-1250] | AST (U/l) | 71.0±62.0 [21-352] | Hb (g/dl) | 11.4±2.4 [5.3-14.9] | GGT (U/l) | 2362.8±2476.4 [13-8753] | TP (g/dl) | 5.4±0.9 [4.0-7.1] | Urea (µmol/l) | 1636.9±562.5 [724.5-3253.3] | ALB (g/dl) | 2.5±0.2 [2.3-3.1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino Acids (nmol/ml) | | | | | | | | | | | | | | |
| Thr | 48.4±31.5 [12.8-167.3] | Asp | 6.7±1.9 [1.5-10.3] | Val | 138.8±57.8 [18.2-315.2] | Ser | 64.2±27.8 [23.8-147.9] | Met | 18.1±12.5 [3.8-58.4] | Asn | 28.4±11.3 [13.0-64.7] | Ile | 44.0±18.3 [19.7-108.2] | Gln | 99.5±70.4 [15.2-228.6] | Leu | 81.1±33.4 [18.4-166.9] | Gly | 221.9±119.5 [36.5-592.6] | Phe | 39.4±16.2 [1.9-89.0] | Ala | 270.8±140.6 [91.3-667.9] | His | 65.3±30.1 [21.8-168.4] | Tyr | 48.6±28.0 [11.0-137.7] | Trp | 22.9±10.8 [8.1-55.1] | Pro | 145.1±111.2 [26.0-527.2] | Arg | 46.1±20.6 [11.1-101.8] | Cit | 40.7±11.6 [25.5-75.2] | EAA | 545.2±213.1 [176.2-1242.4] | Orn | 23.7±14.2 [10.1-82.8] | TAA | 1735.9±618.7 [604.5-3733.4] | NEAA | 1190.7±443.5 [428.4-2491.0] |
| EAA | 545.2±213.1 [176.2-1242.4] | NEAA | 1190.7±443.5 [428.4-2491.0] |

Table 1. Hematological, biochemical states and serum concentrations of amino acids in 28 newborn calves

| Ht | RBC | Hb | TP | ALB | AST | GGT | NH₃ | Urea |
|---|---|---|---|---|---|---|---|---|
| Asp | *** | *** | *** | *** | | | | |
| Thr | *** | *** | *** | *** | * | | |
| Ser | * | * | * | * | * | | |
| Asn | ** | * | * | * | | | |
| Gla | *** | *** | *** | *** | | | |
| Gin | *** | *** | *** | *** | | | |
| Gly | *** | *** | *** | *** | | | |
| Ala | *** | *** | *** | *** | | | |
| Val | *** | *** | *** | *** | * | * | * |
| Met | *** | *** | *** | *** | | | |
| Ile | * | * | * | * | * | * | |
| Leu | *** | *** | *** | *** | * | * | |
| Tyr | * | * | * | * | * | |
| Phe | * | * | * | * | | | |
| Lys | * | * | * | * | | | |
| His | * | * | * | * | * | * | |
| Trp | * | * | * | * | * | | |
| Arg | * | * | * | * | | | |
| Pro | *** | *** | *** | *** | || |
| Cit | * | * | * | * | | | |
| Orn | * | * | * | * | | | |
| EAA | * | * | * | * | | | |
| NEAA | * | * | * | * | | | |
| TAA | * | * | * | * | | | |

Figure 1. The Pearson correlation coefficients (r) between the hematological or biochemical states and serum concentrations of each amino acid, essential amino acid, non-essential amino acid or total amino acid. Gray-colored and white-colored boxes show negative and positive Pearson correlation coefficients, respectively. Statistical correlations were determined as three levels of significance: *p<0.05, ** p<0.01 and *** p<0.001
blood may influence the negative correlation between hematological values and total AA concentrations.

All of free AAs demonstrated positive correlations with ammonia, but no significant correlation with urea. Many AAs can be metabolized to ammonia, and ammonia is almost immediately converted to urea in the liver urea cycle [1]. The activity of the urea cycle in the liver increases after approximately 2 weeks of age [8], with elevated urea levels dependent on growth rates in calves [9]. During the period following birth, transportation of free AA from colostrums may cause retention of ammonia in the blood, due to the undeveloped urea cycle in the liver.

Glu recorded the highest correlation (r=0.88) with GGT. This indicates that the serum state of Glu is significantly influenced by colostrum intake immediately after birth [10]. Serum levels of Glu already increased 2.5 to 4 times in the 2 hours after birth [3]. This change is also dependent on the rate of Glu metabolism by glutaminase within the intestinal membranes [11]. Colostrum intake is thought to be a metabolic trigger to convert Gln to Glu [11]. Gln is one of the major AA transferred from the mother's blood in utero [10]. Glu reportedly showed a distinct decrease with colostrum intake on the first day of life, despite being the major free AA within cow colostrums [3,10]. Serum GGT levels greater than 200 U/l were suggested to relate to sufficient IgG1 levels above 1000 mg/dl [12]. According to our linear regression for the relationship between GGT and Glu, serum concentrations of Glu should be greater than 30.1 mmol/l in healthy calves suckling sufficient colostrums. Glu is an essential metabolite for the newborn calves and may be central to muscle development and structure [13]. Additionally, Glu may play support roles for the activation of immunological systems [1]. Our data supports that supplements of Glu for newborn calves with insufficient suckling of colostrum are beneficial.

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