# The effects of training and gelatine supplementation on plasma amino acid profile in resting horses

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## Introduction

The equine body contains ~180 g protein/kg (*Webb* and *Weaver* 1979, *Martin-Rosset* 2005). The plasma protein can be calculated at 3 g/kg body weight (52 ml plasma volume, 60 g total protein/l; *Coenen* and *Vervuert* 2003). Consequently the plasma protein and the additional free amino acids (AA) represent a small quantity of total body protein and total AA. But the changes in plasma AA in several conditions like fast, pregnancy, exercise (*Buraczewska* et al. 1972; *Silver* et al. 1994, *Zicker* and *Rogers* 1994; *Assenza* et al. 2004; *Bergero* et al. 2005, *Vervuert* et al. 2005) show the sensitivity of the plasma AA pool against external or internal changes. In general it is suggested that exercise do not change protein requirement and an increased protein supply to exercising horses is disadvantageous (*Graham-Thiers* et al. 2001). On the other hand, exercise changes the AA homeostasis particular in young horses and on the other hand exercise induces a severe load of the joint associated tissues. Studies in other species show benefits of a gelatine intake on the function of stressed cartilage.

Objectives of the present study were to investigate the plasma amino acid profiles during a training period and the modification by gelatine supplementation.

## Material and Methods

Standardbred trotters (n=13; 403±41 kg BW) were fed a hay-concentrate diet (2 meals per day) enriched by soy bean meal and soy bean oil (104-127 MJ DE, 730-883 g digestible protein/day; free access to water). Seven horses were supplemented by 60g hydrolyzed gelatine/day. The remaining 6 horses served as controls.

The gelatine supplement contributed <5 % of the total daily AA consumption, with the exception of proline (8 %), glycine (15.6 %), alanine (7.1 %), arginine (8.5 %). Hydroxyproline absent in the other feeds unless gelatine

All horses underwent a 10 weeks training period on a high speed treadmill (3 % incline) with 3 standardized exercise tests (SET, start, middle, end; 5 or 6 steps, 4 min each; starting by 5 m/sec, 1 m/sec increment/step) and every second day in alternate order an interval exercise session (3 x 5 min trot at $V_2$-speed to achieve 2 mmol lactate/l blood under defined SET conditions – plus 3 x 4 min sprints at $V_{10}$) or an 45 min lasting endurance run (at $V_2$). Blood samples were obtained at rest once per week at 2 hour after feeding (effect of training). A second sampling procedure in hourly intervals covered the time postprandial. The free AA were analyzed by a HPLC system. Results are presented as mean ± standard deviation. Analyzes of variance for repeated measurements was used for statistics (Statistika®) including the LSD-test.

## Results

Most AA showed an increase after diet change and start of the training period (Table 1). The maxima were reached predominantly during the first 4 weeks. Glutamate was the only AA which decreased during the training. Some AA including proline, taurine and glycine were unaffected by time of training. Within 2 week the gelatine supplementation induced significant positive changes for glycine and proline. Over 8 hours postprandial glutamic acid, cystine, methionine and tryptophan were unchanged while most AA showed a curve with the highest values at 120 min after feeding (Table 2). During the postprandial period existed significant differences in plasma AA in favour for the gelatine supplemented horses (at >2 samplings) for glycine, and proline; further differences are detectable although the contribution of gelatine to total AA intake was small (e.g. ornithine, alanine, leucine, serine and valine).

**Table 1** Free amino acids (µmol/dl) in blood plasma during 10 week of training (sampled weekly 2 h postprandial at rest; N=13 unless others are indicated in brackets).

| AA with sign. changes during training | | | | | AA without time effect[2] | |
|---|---|---|---|---|---|---|
| ° | mean[1] ± SD | change | max | week | ° | mean ± SD |
| Ala | 20.5 ± 4.1 | ⇧ | 29.7 | 4 | Asp | 1.1 ± 0.5 |
| Arg | 11.3 ± 3.0 | ⇧ | 17.5 | 7 | Gln | 44.6 ± 6.0 |
| Asn | 4.2 ± 1.8 | ⇧ | 6.4 | 7 | Gly[3] | 61.3 ± 4.7(6) |
| Cys | 0.1 ± 0.2 | ⇧ | 0.8 | 10 | His | 11.5 ± 1.0 |
| Ileu | 7.7 ± 2.2 | ⇧ | 10.7 | 2 | Orn[3] | 8.8 ± 1.2(6) |
| Leu | 9.8 ± 2.9 | ⇧ | 12.1 | 2 | Pro[3,4] | 14.3 ± 2.5(6) |
| Lys | 9.8 ± 3.8 | ⇧ | 13.8 | 4 | Tau | 6.2 ± 0.7 |
| Meth | 3.2 ± 0.6 | ⇧ | 4.2 | 2 | Thre | 13.5 ± 1.9 |
| Phe | 7.3 ± 1.2 | ⇧ | 8.6 | 9 | **AA with sig. effect of gelatine-supplementation** | |
| Ser | 27.9 ± 6.6 | ⇧ | 35.6 | 4 | | |
| Try | 6.6 ± 1.9 | ⇧ | 9.2 | 4 | ° | mean[5] ± SD |
| Tyr | 7.4 ± 1.8 | ⇧ | 9.4 | 4 | Gly C | 62.8 ± 5.5(6) |
| Val | 20.3 ± 3.2 | ⇧ | 25.2 | 4 | Gly G | 88.6 ± 9.9(7) |
| Gln | 4.8 ± 1.8 | ⇩ | | | Pro C | 14.0 ± 2.5(6) |
| | | | | | Pro G | 19.8 ± 6.2(7) |
| | | | | | Orn C | 8.9 ± 1.2(6) |
| | | | | | Orn G | 10.3 ± 1.6(7) |

[1] at start of the training period; week gives the week during the training with the observed maximum; [2] mean over week 0-10, [3] control group only
[4] week 0 excluded, sign. lower in comparison to the following weeks;
[5] by reason of intitial changes data for week 0 and 1excluded

## Discussion

The horses responded on the diet during the first weeks of training by an increase in most AA. This response was superimposed by the supplemented gelatine for those AA which dominate in gelatine. As the sampling occurred 2 h after fee-

ding and >15 h after the last exercise, an echo effect from the exercise can be excluded. Considering the high protein intake it´s unlike that there was a shortage in AA in any of the horses regardless the treatment. The horses were adapted to exercise on the treadmill and in that way, the basic stimulation of the digestive tract by exercise was present at start of the training period. In so far it can be concluded that the diet change and the gelatine supplementation delivered AA which are in excess available and used for energetic purposes. An increased need for specific AA exists e.g. for alanine serving the ammonia elimination from the muscle cells. Interestingly some AA were unchanged during the feeding/training period. Taurine belongs to these group although the function of the heart muscle is linked to the availability of taurine and one could expect that training associated adaptations in heart function modify taurine turnover.

**Table 2** Plasma free amino acids (µmol/dl) during 8 h postprandial.

| amino acids unaffected by time postprandially and supplementation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Glu 3.6±1.8; Cys 0.5± 0.5; Meth 3.1±0.8; Try 7.0±1.9 | | | | | | | | | | | |
| amino acids affected by time postprandial | | | | | | | | | | | |
| time, h | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | SD[1)] |
| Arg | | 9.17 | 15.7 | 16.6 | 15.4 | 14,0 | 11.7 | 11.2 | 10.4 | 9.1 | 3.9 |
| Asp | | 2.6 | 5.6 | 6.6 | 6.4 | 5.7 | 4.9 | 4.3 | 4.0 | 3.2 | 1.7 |
| Gln | | 33.6 | 43.7 | 49.2 | 48.2 | 48.0 | 46.7 | 44.1 | 41.0 | 36.5 | 7.6 |
| His | | 8.0 | 10.9 | 11.6 | 11.8 | 11.2 | 10.7 | 10.5 | 9.7 | 8.7 | 2.0 |
| Ileu | | 6.4 | 9.8 | 10.0 | 9.7 | 8.7 | 7.5 | 7.0 | 6.4 | 6.1 | 2.2 |
| Lys | | 6.2 | 12.4 | 14.2 | 12.6 | 10.7 | 8.6 | 7.6 | 6.9 | 6.6 | 3.7 |
| Phe | | 6.6 | 8.9 | 9.3 | 8.9 | 8.3 | 7.7 | 7.4 | 7.6 | 6.6 | 1.6 |
| Tau | | 5.0 | 6.0 | 6.4 | 6.1 | 5.7 | 5.4 | 5.2 | 5.1 | 5.0 | 0.8 |
| Thr | | 8.9 | 12.8 | 14.48 | 13.8 | 13.0 | 11.6 | 11.2 | 10.1 | 8.9 | 2.8 |
| Tyr | | 6.2 | 8.6 | 8.9 | 8.8 | 8.1 | 7.2 | 6.7 | 6.2 | 5.9 | 1.6 |
| amino acids affected by time x feeding (C: control vs. G: gelatine supplement) | | | | | | | | | | | |
| Ala | G | 15.4 | 25.0 | 29.5 | 29.9 | 28.6 | 25.6 | 22.5 | 20.3 | 17.2 | 6.1 |
| | C | 16.5 | 23.8 | 24.6 | 25.5 | 23.6 | 22.8 | 22.4 | 18.6 | 16.9 | 5.3 |
| Gly | G | 76.6 | 91.7 | 99.4 | 92.3 | 94.1 | 89.3 | 84.3 | 82.6 | 78.1 | 10.7 |
| | C | 57.3 | 63.1 | 67.5 | 71.1 | 61.1 | 59.0 | 60.7 | 59.3 | 58.2 | 10.4 |
| Leu | G | 9.8 | 13.5 | 14.7 | 13.2 | 12.8 | 10.7 | 9.6 | 8.9 | 8.4 | 2.9 |
| | C | 7.9 | 12.4 | 11.3 | 11.4 | 9.7 | 8.7 | 8.6 | 8.3 | 8.0 | 2.8 |
| Orn | G | 7.2 | 9.9 | 12.8 | 11.6 | 11.1 | 10.5 | 9.1 | 9.2 | 7.7 | 2.6 |
| | K | 6.4 | 8.4 | 9.2 | 9.6 | 9.6 | 8.9 | 8.5 | 7.2 | 6.8 | 2.0 |
| Pro | G | 8.7 | 19.7 | 19.6 | 19.9 | 18.7 | 15.2 | 14.3 | 11.5 | 10.7 | 5.4 |
| | K | 7.9 | 15.2 | 14.7 | 14.9 | 12.9 | 12.3 | 12.7 | 9.4 | 10.1 | 3.8 |
| Ser | G | 26.2 | 32.9 | 37.2 | 35.5 | 34.9 | 33.7 | 31.5 | 32.0 | 29.1 | 4.2 |
| | K | 24.4 | 31.5 | 33.5 | 33.4 | 32.4 | 31.0 | 32.5 | 30.2 | 27.7 | 4.1 |
| Val | G | 21.7 | 26.2 | 28.4 | 27.0 | 26.1 | 24.2 | 22.4 | 21.8 | 19.2 | 4.7 |
| | K | 16.9 | 21.9 | 21.2 | 22.4 | 19.7 | 18.7 | 18.0 | 17.8 | 16.9 | 4.3 |

The gelatine supplementation creates a persistent significant effect in the cartilage related AA glycine, proline and alanine. So far, the AA addition by gelatine to the total AA intake is effective and the AA should be available for tissue specific demands. The postprandial changes indicate that independent of the long lasting modification of AA homeostasis by gelatine, during the absorptive phase of digestion further AA are rapidly available from this source. Taurine can be taken as an indicator for absorption of AA from the basic diet as taurine is absent in gelatine. The plasma levels show a peak at 2 h after feeding. The protein absorption follows a comparable curve like glucose absorption from starch digestion (*Vervuert* et al. 2005). The elevated supply of AA by gelatine (Table 2) produce maxima in plasma free AA within the same window of time and can be valuated as highly digestible in the small intestine. Hydroxyproline is presented by gelatine at high amount but not detectable in plasma. The changes in plasma proline under control condition and after feeding gelatine indicate that plasma proline is "fed" by hydroxyproline in gelatine, this AA probably is converted to proline throughout the absorption.

## Conclusion

Gelatine is a cheep well purified product from the processing of connective tissue from ruminants and pig. Rather small amount are efficient to supply those AA to the AA pool which are of great interest for cartilage metabolism. So far it is justified to consider gelatine as a possible cartilage supporting AA source. Further studies should be focussed on the protective capacity.

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