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# The weaning-related changes in amino acids status of blood plasma in piglets

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**Abstract:** The aim of this study was to investigate changes of hormones and plasma amino acids in piglets subjected to weaning procedure. Twelve male piglets (Large Polish White breed) were used in the experiment. Piglets were held with their mothers from birth up to the 30th day of neonatal life, and on that day weaned, and housed individually for 5 days. Selected plasma amino acids and insulin-like growth factor-1 (IGF-1), growth hormone (GH) and cortisol concentrations in blood serum of piglets before and after weaning were analyzed.

IGF-1 concentration showed a tendency to decrease in 35-day-old animals. There was no change in the values of cortisol and GH concentration between not-weaned and weaned piglets. Cysteic acid plasma concentration was significantly higher in piglets after weaning, but the levels of taurine, threonine, glutamine, alanine, valine, leucine, ornithine were lower in 35-day-old piglets. In piglets after weaning there was the tendency to an increase in plasma concentration of arginine, glutamate and glycine.

Analysis of plasma concentrations of other amino acids showed a tendency to lower values in piglets at the age of 35 days of postnatal live.

The mechanisms of the homeostatic control of amino acids metabolism in blood plasma were not able to sustain an adequate range of their concentrations after weaning. The obtained results of lowered levels of the majority of amino acids indicate that inhibited growth rate and body weight gain of weaned piglets might be the cause of the observed effects.

**Keywords:** blood plasma amino acids, hormones, postweanig time, piglets

## INTRODUCTION

The transition from maternal milk to dry food results in a period of starvation which, in association with the absence of the dam, impairs energy status and thermoregulation, as shown by behavioural and neuroendocrine changes [1, 2]. The low nutrient intake during the first days after weaning is the main cause of reduced growth [3, 4]. Early weaning is often accompanied by diarrhoea [5]. The young weaned pig is abruptly subjected to a radical change in diet, coupled with the lack of maternally derived serum immunoglobulins and the withdrawal of the locally protective elements of milk [6]. The process of weaning causes immediate and substantial changes in the behaviour of piglets, with these changes being affected by both the age of the piglets at weaning and by individual characteristics, including suckling behavior and relative weight at weaning [7]. There appears to be general agreement that food intake during the first few weeks of postnatal life has a determining influence on the subsequent growth and size of animals. Abrupt modification of the environment and feeding at weaning connected with inappetance reduce weight gain and induce immediate or subsequent adaptive changes in the behavior and physiology of piglets, compared with those suckling their mother [8]. Several studies have shown that the age related change of digestive enzymes is an adaptation to the dietary alteration in piglets at weaning. These changes occur according to changes in the quantity of dietary protein, fat and carbohydrates [9, 10]. All these factors lead to systemic growth inhibition induced by strongly reduced food consumption and negative changes in the intestine structure [11].

Moreover, the piglets are a very suitable experimental model for investigations concerning human nutritional studies or physiology and pathology of the human offspring because there are many similarities in the physiology and anatomy of the gastrointestinal tract [12, 13].

The effects of both stress and low feed intake influence hypothalamic-pituitary-adrenal axis (HPA) hormone responses at weaning [8]. Weaning induces changes in a number of hormones involved specifically in the response to the stress of weaning, e.g. HPA hormones, and sympathetic nervous system (SNS) hormones, and in the energy metabolism – thyroid and hormones of the somatotropic axis.

The aim of this study was to investigate changes in the status of hormonal and blood plasma amino acids in piglets subjected to weaning procedure.

## MATERIALS AND METHODS

This study was approved by the Local Ethics Committee for Animal Experiments of University of Life Sciences in Lublin, Poland.

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### Experimental design

Twelve male piglets (Large Polish White breed) were used in the experiment. Animals were divided in 2 groups: 30 (n=6) and 35 (n=6) days old piglets. Animals were clinically healthy. Piglets were held with their mothers from birth up to the 30$^{th}$ day of neonatal life, weaned at this time, then housed individually for 5 days in pens under standard rearing conditions (controlled and constant temperature and humidity, and a controlled 12:12-h light-dark cycle) with free access to fresh water. Sows during pregnancy and lactation, and their weaned youngsters, were fed twice a day with standard commercial diets for sows and piglets, respectively.

### Blood sample collection

None of piglets was fasted before blood collection. Blood samples were obtained from piglets by standard venipuncture. One part of the blood samples after clotting, and other part were collected in heparinized tubes and centrifuged at 3,000 x g for 15 min. The obtained blood serum and plasma samples were stored at -25°C until analysis. The blood plasma samples for the determination of the concentrations of amino acids were analyzed with the use of an ion-exchange chromatography on an INGOS AAA 400 amino acid analyzer (Ingos, Czech Republic).

Serum growth hormone GH concentration was assessed with the use of Porcine Growth Hormone Enzyme-Linked Immunosorbant Assay (ELISA; Diagnostic System Laboratories, Inc., Webster, TX, USA). Serum cortisol and IGF-1 concentration were assessed using Cortisol Elisa Kit (IBL Gesellschaft für Immunchemie und Immunbiologie, Hamburg) and IGF-I Elisa Kit (R&D, USA), respectively. Standard curves were generated using growth hormone, cortisol or IGF-1 provided with the kit, and the absolute concentrations in the serum were obtained from these curves. The determination of serum hormonal concentrations was obtained using Benchmark Plus microplate spectrophotometer supplied with Microplate manager Software Version 5.2.1. (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

### Statistical analysis

All results are expressed as means ± SEM (standard error of the mean). Differences between means were tested with the One Way ANOVA. Normal distribution of data was examined using the W. Shapiro-Wilk test, and equality of variance tested by the Levene test. If there was a lack of normal distribution and/or unequal variance of data, the Mann-Whitney U-test was used to test the differences between means. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out with the use of STATISTICA 6.0 software (StatSoft Polska, Krakow, Poland).

## RESULTS

There were no statistically significant differences in weight between 30 and 35-day-old animals.

### Insulin-like growth factor-1 (IGF-1), growth hormone (GH) and cortisol serum concentrations.

Insulin-like growth factor-1 (IGF-1) concentration showed a tendency to decrease in 35-day- old animals (Table 1). Moreover, changes in the values of cortisol and GH concentration in piglets investigated after weaning were not observed (Table 1).

**Table 1** Concentrations of growth hormone (GH), cortisol and insulin-like growth factor – 1 (IGF-1) obtained in blood serum of unweaned piglets (at the age of 30 days of life) and at the age of 35 days (after weaning at 30th day of life).

| | 30 day (unweaned) | 35 day (after weaning) |
|---|---|---|
| IGF1 [ng/ml] | 83.16 ± 4.20 | 75.03 ± 5.94 |
| GH [ng/ml] | 5.24 ± 0.26 | 5.18 ± 0.32 |
| Cartisol [ng/ml] | 51.63 ± 6.40 | 52.85 ± 6.99 |

Differences between the mean values of groups were not significant at P≤0.05

### Plasma amino acids concentrations

Cysteic acid plasma concentration was significantly higher in elder piglets (Table 2). The level of taurine, threonine, glutamine, alanine, valine, leucine, ornithine was significantly lower in 35-day-old animals (Table 2). The tendency to increase in plasma concentration of arginine, glutamate, glycine in piglets after weaning was observed (Table 2). The analysis of plasma concentrations of all the other amino acids exluding lysine and histidine showed a tendency to lower values in piglets at the age of 35 days of postnatal live (Table 2).

**Table 2** Free amino acids concentration (nmol/mL) obtained in plasma of unweaned piglets (at the age of 30 days of life) and at the age of 35 days (weaned at the age of 30 days of life).

| Amino acid | 30 day (not weaned) | 35 day (after weaning) |
|---|---|---|
| essential: | | |
| Arginine | 74.33 ± 20.39 | 132.83 ± 13.43 |
| Histidine | 77.50 ± 7.64 | 73.17 ± 4.74 |
| Isoleucine | 94.33 ± 13.46 | 71.67 ± 10.68 |
| Leucine | 172.50 ± 9.92 | 131.17 ± 13.14 * |
| Lysine | 163.17 ± 7.26 | 165.33 ± 17.16 |
| Methionine | 29.83 ± 3.97 | 24.33 ± 5.54 |
| Phenyloalanine | 82.83 ± 5.91 | 68.67 ± 6.57 |
| Threonine | 154.00 ± 8.73 | 78.33 ± 12.19* |
| Tryptophan | 40.17 ± 3.41 | 32.00 ± 1.46 |
| Valine | 212.33 ± 18.51 | 152.50 ± 13.24* |
| non essential: | | |
| Alanine | 789.17 ± 46.63 | 560.33 ± 23.81* |
| Aspartate | 16.67 ± 0.80 | 12.67 ± 1.82 |
| Alpha-amino-butyrate | 18.00 ± 3.57 | 9.33 ± 2.88 |
| Citrulline | 69.33 ± 7.08 | 48.83 ± 11.51 |
| Cystatione | 6.00 ± 1.44 | 3.67 ± 0.61 |
| Cysteic Acid | 3.67 ± 0.76 | 6.17 ± 0.60 * |
| Glycine | 528.67 ± 79.78 | 547.17 ± 51.75 |
| Glutamate | 90.17 ± 8.35 | 104.83 ± 20.38 |
| Glutamine | 216.33 ± 9.04 | 165.83 ± 16.04* |
| Ornithine | 137.50 ± 9.01 | 65.83 ± 5.94* |
| Proline | 892.00 ± 92.63 | 787.67 ± 73.71 |
| Serine | 125.50 ± 12.45 | 95.33 ± 7.89 |
| Tyrosine | 113.17 ± 9.01 | 90.83 ± 10.52 |
| Taurine | 179.33 ± 11.42 | 94.83 ± 7.49* |

* – differences significant at P≤0.05

## DISCUSSION

Our results showed that the negative effect of weaning on body mass gain during 5 days was not intensive. Body mass gain is a results of complex processes, and requires the coordinated action of several hormones. Nutritional status has a strong

influence on the expression/secretion of a variety of growth-related hormones, including somatomedines, GH, thyroid hormones, and glucocorticoids [14]. The major participant in physiological growth is GH which influences the whole metabolism of the developing organism. Our piglets showed a tendency to a decrease in the serum concentration of GH. This decreased activity was followed by less stimulation of the liver and other tissues to secrete somatomedines. One of them, IGF-1 is a key in muscle and bone growth, and also stimulates amino acids uptake and protein synthesis in muscle and other tissues, including bones. Circulating IGF-1 is mainly produced in the liver via regulation not only by GH but also by the diet. IGF-1 is essential for longitudinal growth, and can be considered as an important controller of the intestinal absorption of nutritional components. In our study, IGF-1 showed a tendency to decrease in 35-day-old piglets weaned on the 30$^{th}$ day. The nutritional status exerts a direct influence on circulating IGF-1 level which is closely related to the energy intake and hence to growth rate [8, 14, 15]. The weaning-related endocrine change is a decreased level of cortisone, although this reduction is considerably lower in the later than earlier weaned animals [16]. The transient increase in cortisol excretion in weanlings may be caused by both emotional distress and acute food deprivation [1]. This probably reflects physiological responses to insufficient energy intake after weaning, as reflected also by changes in thermoregulatory behavior. Our results showed that weaning did not influence the level of cortisol.

Changes in quantity of dietary protein, fat and carbohydrates are also followed by substantial changes in amino acids metabolism [5]. Arginine, glutamine, glutamate, proline, aspartate, asparagine, ornithine, and citrulline are interconvertible via complex interorgan metabolism in most mammals, including the pig. The small intestine, kidney and liver are the major sites for their metabolism, with cortisol being a key regulatory hormone [17]. The abundance of glutamine in sow's milk is of nutritional and physiological significance, compensating the low concentration of arginine in neonatal diet. Moreover, there is little uptake of proline by the pig small intestine, and enteral provision of large amounts of proline from the sow's milk is crucial for compensating for the deficiency in arginine [17]. Essential amino acids are not synthesized by mammalian tissues, and the major source of these for muscle mass is dietary protein in the normal stage. In our study, there was observed a significantly lower concentration of these group of exogenous amino acids. The metabolism of amino acids, amines and neurotramsmitters is connected with the metabolism of carbohydrate, single or multiple enzyme steps for alanine, aspartate, glutamate, glucose, glutamine, glycine, histidine, leucine, phenylalanine, polyamine, tryptophan, taurine, and tyrosine metabolism [18]. The fasting state of our piglets after weaning led to changes in intermediary metabolism in the liver, induced the decrease of leucine, taurine, alanine, glutamine, aspartate, phenylalanine, tryptophan and tyrosine in blood plasma. The liver is also the primary site for the urea cycle, connecting pathway of ornithine and citrulline metabolism [19]. Both of them were lowered in presented piglets after weaning. This may indicate that the entire metabolism of piglets decreased, caused by lack of appetite. The decreased concentration of branched-chain amino acids, leucine and valine, and the tendency to lower the values of isoleucine in blood plasma of the presented post-weaned piglets may indicate lower transamination of these amino acids in skeletal muscle and reduced metabolic processes in the liver. Their respective branched-chain keto acids transported to the liver for further metabolism were finally decreased [19]. Moreover, the concentration of methionine, amino acid involved in polyamine synthesis was also decreased and may be linked with lower histological development of enterocytes of weaned piglets. However, the level of arginine showed a tendency to increase in piglets after weaning. This could be caused by the adequate activity of enzymes located in the intestinal mucosa playing a significant role in endogenous arginine synthesis. The complete pathway of its intestinal tract is observed in weaned pigs [20].

In the present study, the tendency to a higher level of glutamate in piglets after weaning was observed. It can be synthesized from proline and branched-chain amino acids, and from glutamine, the concentration of which was decreased in the elder piglets in our study. Moreover, enteral and plasma glutamine serve as precursors for the intestinal synthesis of citrulline in pigs and other mammals [20]. It is not surprising that the level of citrulline showed a tendency to decrease in our post-weaned piglets.

The concentration of modulatory neurotransmitters, such as glutamate and glycine, was unchanged after weaning in 35-day-old piglets. Moreover, glutamate remained unchanged, although it participates in two cycles: glutamate-glutamine and leucine-glutamate, whereas glutamine and leucine, amino acids playing the role in brain nitrogen balance, was decreased. The long-lasing state of undernutrition during weaning or intentional starvation may influence synaptic activity [18].

The present study indicates that unintentional starvation during weaning, inadequate or insufficient diet or protein supply and intake, necessary for normal development and physical activity, may influence the gain in body mass by the changes in the status of plasma amino acids in piglets, and probably also in humans. All these changes also influence enzymatic ability, function, and histomorphometry of the digestive tract [21]. The lack of changes in concentration of hormones playing a pivotal role in general growth of piglets may indicate that there is amino acids regulatory effect on the whole body and muscle protein synthesis. Decreased level of many amino acids is perhaps determined by a decrease in tissue metabolism as a response, not only to hormone stimulation, but also to the nutrient supply. A significantly lower level of exogenous and a strong tendency to lower concentrations of other amino acids in blood plasma of piglets in the present study was also connected with the stressful state of the weaning. The significant increase of cysteic acid needs further study.

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