INTRAPERITONEAL ADMINISTRATION OF TRYPTOPHAN DECREASES LIQUID DIET INTAKE IN EARLY WEANED PIGLETS

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Abstract


The aim of the present study was to investigate the effect of intraperitoneal tryptophan (Trp) administration on liquid diet consumption in early weaned piglets. Furthermore, the effects of two chemical forms of the amino acid were compared.

Single doses (100 mg.kg⁻¹) of L-tryptophan (480 mmol.kg⁻¹) or L-tryptophan methyl ester (392 mmol.kg⁻¹) (Sigma, USA) were i.p. administered at 07.00 h to 2 groups of 5 piglets each at the age of 3, 9, 12, 14 and 16 days. The piglets were weaned on d 1, housed individually in cages and offered a commercial diet (Selasan) for suckling from feeding bottles between 06.00 and 22.00 h 9 times a day at 2-h intervals with an 8-h break at night. Control piglets (n=5) were left intact.

Compared with intact controls, L- Trp decreased the relative sizes of individual meals in piglets aged 3 d only 5 h (P < 0.02) after its administration, in the 9-d-old ones 1, 3 and 5 h (P < 0.05; P < 0.01; P < 0.05, respectively), and in the 16-d-old piglets 3 h (P < 0.01) later. No significant differences between control and L-Trp-treated animals occurred on days 12 and 14.

L- Trp methyl ester decreased the diet consumption of piglets at all ages as follows: in 3-, 9- and 16-d-old piglets 3 h (P < 0.05; P < 0.05; P < 0.01, respectively) post-injection, in the 12-d-old animals 1 h (P < 0.01), 3 h (P < 0.001) and 7 h (P < 0.05), and in the 14-d-old piglets 1 h (P < 0.01) and 3 h (P < 0.01) after L-Trp methyl ester administration. In 12- and 14-d-old piglets its effect was also reflected in 24-h diet intake that was significantly decreased. Similar differences in diet consumption were found when the relative sizes of individual meals were compared to the first morning consumption, i.e. 1 h before Trp treatment.

In conclusion, Trp did suppress food consumption in early weaned piglets as soon as on day 3 after birth, indicating that the aminostatic component of their food intake regulation is functional at this age. At the same time it was found that this effect of L-trp methyl ester (the actual dose of which was smaller than that of L-Trp) occurs earlier, is more profound and lasts longer than that of L-Trp.

Suckling, voluntary diet intake, L-tryptophan, L-tryptophan methyl ester

Local tryptophan concentration influences rapidly the synthesis and release of serotonin by brain neurons (Fernstrom 1990; Sharp et al. 1992), and the close dependence between the central serotoninergic system and precursor of serotonin (5-HT), the amino acid tryptophan (Trp) has been repeatedly demonstrated (e.g. Leathwood 1987b, 1988; Fernstrom and Fernstrom 1995). Trp availability and its interaction with large neutral amino acids (LNAA) in the internal environment of the body modulates functioning of this system. Its high content in the diet increases the concentration of serotonin in the brain of rats (Fernstrom and Wurtman 1971; Yokogoshi et al. 1987), primates (Leathwood and Fernstrom 1990), and turkeys (Lee and Britton 1982). Its high dietary concentration relative to other LNAA elicits an increase in brain serotonin synthesis in rats (Leathwood 1987a), chicks (Harrison and D’Mello 1986) and laying hens (Laycock and Ball 1990). It also reduces glycemia, possibly via glucose-mediated increased release of insulinovertic polypeptide (Porter et al. 1994) in early weaned piglets. On the other hand, limited intake of Trp in human diet results in a decreased plasma concentration to 19 % of its original concentration, and to changes in nutritional selection of
proteins (Young et al. 1988). Likewise, limited availability of Trp in the diet due to limited access to food (2 times 1 h per day), significantly decreases concentration of this amino acid in blood plasma of broiler chicks (Baranyiová et al. 1982). Limited availability of Trp in the diet directly affects plasma free amino acids and hypothalamic serotonin in finishing pigs (Henry et al. 1992). The dietary balance of tryptophan in early weaned piglets affects the rate of protein synthesis in various tissues (Cortamira et al. 1991).

When feeding diets with higher tryptophan content, feed intake of rats is inhibited (Ashley and Anderson 1975). Similar feed intake depression was observed in chicks after oral administration of tryptophan (Lacy et al. 1982). However, feed intake was also reduced by ingestion of tryptophan-deficient diet in piglets (Sève et al. 1978), but also when a diet with Trp:LNAA imbalance was fed to finishing pigs (Henry et al. 1992).

After intraperitoneal administration of tryptophan, various nutritional responses were obtained in several species. Rats did not show changes in food consumption (Weinberger et al. 1978; Peters and Harper 1984), or their food intake decreased only slightly (Fernstrom and Wurtman 1972) whereas other authors confirmed the suppressive effect of tryptophan on food intake and selection of nutrients in laboratory rodents and birds (Morris et al. 1987; Pinchasov et al. 1989). When comparing the procedures in these papers we found that in some studies L-tryptophan was employed (Weinberger et al. 1978; Peters et al. 1984) whereas in others L-tryptophan methyl ester (Lacy et al. 1986; Morris et al. 1987) was administered. In several papers no specification of tryptophan was given (e.g. Pinchasov et al. 1989).

The aim of this experiment was to study the development of feeding responses to i. p. administration of this amino acid alone, as it was shown earlier (Baranyiová et al. 1985, 1987; Baranyiová and Holub 1989) that after an i. p. administration of a 20 amino acid solution (with tryptophan included) the aminostatic component of feed intake regulation in piglets begins to come into play in the weaning period. Furthermore, with respect to the above-mentioned differences we compared the effects of both tryptophan preparations on diet ingestion of piglets.

### Materials and Methods

Ten (crossbred Large White x Landrace) littermate piglets (7 females and 3 males) were kept under conventional farm conditions during day one after birth (with approximately 24 h colostrum suckling available to them), then removed from their clinically healthy mother, and transferred to the laboratory. They were weighed, placed individually in cages, and reared in a thermoneutral environment using warming floor pads (Holub 1964, 1968; Kotrbáček et al. 1979; Baranyiová and Holub 1989).

Piglets were offered the Selasan milk replacer diet reconstituted in water and warmed to 38-40 °C for suckling ad libitum from a feeding bottle nine times a day, from 6.00 to 22.00 h followed by an 8 h night pause. Diet intake was measured by weighing the bottle before and after each feeding of each piglet. The piglets were weighed daily prior to first feeding at 6.00 h.

The effects of single intraperitoneal doses of 100 mg.kg⁻¹ (1% solution in physiological saline) L-tryptophan (480 mmol.kg⁻¹) and L-tryptophan methyl ester (392 mmol.kg⁻¹) (Sigma, USA) were studied in groups of 5 piglets each on days 3, 9, 12, 14 and 16 after birth. Five other littermate piglets served as intact controls (in previous studies we did not find differences in diet intake between saline-treated and intact controls; Baranyiová and Holub 1989). Tryptophan injections were invariably given at 07.00 h. The diet consumption was expressed per unit body mass.

Student’s t-test was used to assess the significance of differences between diet intakes in tryptophan-treated and control piglets.

### Results

The relative diet consumption following L-tryptophan administration was decreased compared to the controls in piglets on day 3, 5 h (P < 0.02), on day 9, 1 h (P < 0.05), 3 h (P <
0.01), and 5 h (P < 0.05) later; on day 16, 3 h (P < 0.01) after L-Trp injection. No significant changes in diet intake as against control piglets were observed on days 12 and 14 (Fig. 1).

The relative diet intake after L-tryptophan methyl ester administration (Fig. 1) was decreased at all ages as compared with the controls as follows: in piglets on days 3, 9 and 16, 3 h post injection (P < 0.01; P < 0.001; P < 0.01, respectively), on day 12, 1 h (P < 0.01), 3 h (P < 0.001), and 7 h (P < 0.01), and finally on day 14, 1 h (P < 0.05) and 3 h (P < 0.01) after L-Trp methyl ester administration.

![Graphs showing diet intake](image)

Fig. 1: Relative liquid diet consumption at individual feedings in early weaned piglets. Legend: control (○), L-tryptophan (●) and L-tryptophan methyl-ester (▲) i.p. treatment. The arrows indicate injection times (07.00 h).

When the relative diet intake of experimental piglets was compared to their pre-treatment meal size consumed at 06.00 h, tryptophan administration affected the post-injection meal sizes as follows: L-tryptophan depressed them 3 h later on days 9 (P < 0.05), 14 (P < 0.01); and 16 (P < 0.05) only, see Fig. 1.
On the other hand, administration of L-tryptophan methyl ester resulted in significantly smaller diet intakes compared to their first, pre-treatment consumption on day 9, 3 h (P < 0.001) and 5 h (P < 0.01) later; on day 12, 3 h (P < 0.01), and 7 h (P < 0.05) later, on day 14, 1 h (P < 0.01), 3 h (P < 0.01); and 5 h (P < 0.01) later, and on day 16, 1 h (P < 0.05), 3 h (P < 0.01), and 5 h (P < 0.02) post injection, see Fig. 1.

The more profound and longer lasting food intake depression in piglets treated with L-tryptophan methyl ester than in those treated with L-tryptophan is further documented by the significant differences between these treatments on day 12, 3 h (P < 0.01); and 7 h (P < 0.05) post injection, and on day 14, 5 h (P < 0.02) post injection, see Fig. 1, and also by the decreased whole-day diet consumption on days 12 (P < 0.02), and 14 (P < 0.05), see Fig. 2.

![Fig. 2. Whole-day relative liquid diet consumption in early weaned piglets. For legend see Fig. 1.](image)

Discussion

There is a wealth of data showing that Trp affects feed intake but also feeding behaviour in adult or growing animals (mammals and birds) and man (e.g. Hill and Blundell 1988; Blundell and Halford 1994; Rogers 1995). Results of this study show that tryptophan administered intraperitoneally does exert a suppressive effect on diet intake in piglets as early as on day 3 after birth. This finding extends to younger age the period of operative aminostatic feed intake control in piglets as described in our previous study (Baranyiová and Holub 1989). However, tryptophan was then administered in a solution along with 19 other amino acids (including LNAA), and its dose was about half of that given in this study (though probably high enough to elevate brain serotonin concentration; Leathwood 1988; Holder and Huether 1990). The present findings are not surprising as there is a growing body of data on involvement of serotonin in many vital functions, for example learning and modulation of biological rhythms not only in higher animals but also in invertebrates (Dudai 1988; Schacher et al. 1990; Eskin and Takahashi 1983), and on its multiple roles in fetal and perinatal life (Nock et al. 1978; Ristine and Spear 1984; Manjarrez et al. 1988, a.o.). The present data further support our earlier findings.
showing that in piglets the serotonergic system involved in food intake control is operative after birth; its i. p. administration does suppress feeding and affects feeding behaviour of piglets as soon as 12 h after birth (Baranyiová 1991), also eliciting a general soothing effect similar to that observed in human babies fed 5-HT precursor tryptophan (Yogman and Zeisel 1985).

In this study, Trp was invariably given at the beginning of the light phase of day. In its timing, the feeding regime of our piglets was closely adjusted to that of natural nutrition on the sow when in a cyclical manner only the entire litter (but not an individual piglet) obtains milk after active participation of their mother (e.g. Holub 1994). However, the amount of milk available to each piglet may be limited by a variety of factors, including milk yield of the sow. In our experimental design, the piglets are individually allowed to suckle the diet at predetermined intervals to satiety so that only the piglet’s own regulatory mechanisms determine the amount of diet consumed without interference from the mother or littermates. This makes it a valuable tool to study the timing of development of the regulatory systems.

In the present experiment, differences were observed in the response of piglets to the 2 forms of tryptophan despite the fact that the actual L-Trp methyl ester dose was by 88 mmol lower than that of L-Trp: whereas L-tryptophan methyl ester began to suppress diet consumption significantly 3 h after administration on days 3, 9 and 16, and as soon as 1 h post injection on days 12 and 14, and this effect persisted for 5-7 h between days 9 and 14, that of L-tryptophan did not become significant before 3 h post injection except for day 9. The effect of L-tryptophan methyl ester was also reflected in the diet intake of piglets when expressed as cumulative intake at all 9 feedings per day on days 12 and 14. It can be assumed that these changes are due to differences in the entry of the two tryptophan forms into tissues and cells and to their different metabolic rates. Moreover, there are multiple acceptors for the methyl group (App et al. 1992). The underlying cause of these differences would require further study. Nevertheless the differences may help to explain the contradictory data on the Trp effects of feed intake by different investigators. Therefore when comparing and/or interpreting experimental data, the substances employed should be considered as well. Our data further show that the response to i.p. administration of Trp is changing with advancing age of piglets.

The mechanisms involved in adult food intake regulation are much more complex, involving many interactions such as those between plasma glucose, tryptophan and other, especially the large neutral amino acids, cholecystokinin and a variety of other hormones, neurotransmitters and modulators. Their interplay gives rise to a satiety cascade (Blundell and Halford 1994). However, the accumulating morphological and physiological evidence (e.g., Stephens 1975; Houpt et al. 1977; Holub 1982; Houpt et al. 1983; Ito et al. 1988; Poulat et al. 1992; Baranyiová 1991ab; Holub and Doležel 1994; Baranyiová and Hulinguer 1995, 1996) indicate that also the regulatory systems in newborn mammals are more complex and mature, and capable to function sooner than it was assumed earlier (Hahn 1966). In different phases of postnatal development the relative importance of the individual regulatory systems may change depending also on external factors. The components of the regulatory cascade serve to support the changing homeostasis and harmony of growth (Widdowson 1970).

**Intraperitoneálně podaný tryptofan snižuje konzum tekuté diety u časně odstavených selat**

Vliv intraperitoneální aplikace tryptofanu na konzum tekuté diety byl sledován u časně odstavených selat. Porovnáván byl i účinek dvou chemických forem aminokyselin. Sku-
pinė 5 selat ve věku 3, 9, 12, 14 a 16 dnů byl intraperitoneálně podán vždy v 7 h L-tryptofan (L-Trp) v dávce 100 mg.kg⁻¹ (tj. 480 mmol.kg⁻¹), další skupině (n=5) byl aplikován L-tryptofan methyl ester (100 mg.kg, tj. 392 mmol.kg⁻¹) (Sigma, USA). Selata byla odstavena 1. dne života, individuálně ochovávána v klescích a krmena komerční dietou Selasan z lahví se savičkou mezi 6. a 22. h ve dvouhodinových intervalech s 8-h noční přestávkou. Kontrolní selata (n=5) byla intaktní.

Ve srovnání s kontrolami L-tryptofan snížil konzum diety selat 3. dne za 5 h po jeho podání (P < 0.02), 9. dne za 1 (P < 0.05), 3 (P < 0.01) a 5 (P < 0.05) h, 16. dne za 3 h (P < 0.01).

U selat 12. a 14. dne nebyly rozdíly v konzumu diety mezi skupinou kontrolní a ošetřenou L-Trp. Naproti tomu L-Trp methyl ester snížil konzum diety ve všech věkových skupinách selat, a to následovně: 3., 9. a 16 dne za 3 h (P < 0.05; P < 0.05; P < 0.01), 12. dne za 1 h (P < 0.01), 3 h (P < 0.001) a 7 h (P < 0.05), a 14. dne za 1 h (P < 0.01) a 3 h (P < 0.01) po aplikaci. Tento efekt se navíc projevil u selat snížením celodenního konzumu diety 12 (P < 0.02), a 14 (P < 0.05) dne. Podobně rozdíly v konzumu diety jsme nalezli, když jsme relativní velikost jednotlivých porcí porovnávali s velikostí první ranní porce před podáním tryptofanu.

Tryptofan po i.p. podání snižuje konzum potravy u časné odstavených selat již 3. dne života; aminostatická komponenta regulace příjmu potravy je tedy u nich v tomto věku funkční. Zároveň se ukázalo, že tento efekt nastupuje rychleji, je hubší a přetrvává delší dobu po podání L-Trp methyl esteru.

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