EFFECT OF TRYPTOPHAN ADMINISTRATION ON MELATONIN CONCENTRATIONS IN THE PINEAL GLAND, PLASMA AND GASTROINTESTINAL TRACT OF CHICKENS

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Abstract

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Melatonin is involved in the control of a wide range of physiological processes, including the modulation of gastrointestinal functions. Presence of high levels of this indoleamine in the gastrointestinal tract (GIT) was proved, however, factors influencing its concentrations in this system are still to be elucidated. Therefore we tested effects of oral administration of melatonin precursor tryptophan (L-Trp 150 $mg \cdot kg^{-1}$) on melatonin levels in the gastrointestinal tract (GIT), pineal gland and plasma of broiler chickens. Melatonin concentrations were measured by radioimmunoassay after solvent extraction. Sampling was performed before treatment and within 60 min (application in the middle of the light period) and three hours after the L-Trp treatment (application in the middle of the light and dark period). One hour after L-Trp loading we did not observe changes in melatonin levels. Three hours after L-Trp administration pineal melatonin concentrations rose significantly during the lighttime. Darktime levels in the pineal gland as well as both light- and dark-phase concentrations in the plasma and GIT remained unchanged. These results indicate that an increased availability of melatonin precursor tryptophan may modulate melatonin levels in the pineal gland, plasma and gut of chickens only to a limited extent.

Broilers, enterochromaffin cells, duodenum, ileum, colon, melatonin rhythm

Systemic melatonin is synthesized predominantly in the pineal gland and its pineal and consequently plasma concentrations show a distinct daily rhythm with high concentrations observed during the night. In this way, the melatonin rhythm mediates information on environmental conditions to the organism. It is implicated in control of the circadian system of birds (Gwinner 1989) and seasonal cycles of photoperiodic mammals (Elliott 1976). Besides a role of melatonin in the regulation of biological rhythms it is also involved in control of a wide range of physiological processes (Jankovic et al. 1970; Reiter 1991; Reiter et al. 1997), including the modulation of gastrointestinal functions (Bubenik and Dhanvantari 1989; Bubenik and Pang 1994; Barajas-López et al. 1996; Bubenik et al. 1996). Especially melatonin paracrine and autocrine effects on intestinal motility are of great interest. In vitro melatonin reduces the tone of gut musculature and counteracts the tonic effect of serotonin (5-HT) (Bubenik 1986). Administration of melatonin to intact mice results in a decrease of food transit time (FTT) but melatonin significantly increases FTT in mice bearing an active implant of 5-HT (Bubenik a Dhanvantari 1989). On the

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basis of these results a counterbalancing melatonin and 5-HT system modulating tonus of GIT musculature was hypothesized.

Moreover, L-Trp administration reduces food intake in pigs probably through stimulation of the serotonergic system (Baranyiová 1996). Serotonin influences ingestive behaviour also in broiler chickens (Denbow et al. 1982; Baranyiová 1990), however, a possible relationship between L-Trp and melatonin and its function in regulation of digestion is still to be elucidated.

Melatonin was found in the gastrointestinal tract (GIT) of mouse (Bubenik and Pang 1994), rat (Bubenik et al. 1977; Huether 1994), chicken (Huether et al. 1992; Herichová and Zeman 1996), pigeon (Vakkuri et al. 1985) and Zebra finch (Vant Hof and Gwinner 1996). A source of melatonin in GIT was not definitely identified as yet, although available findings suggest local production in this tissue. Both enzymes involved in melatonin forming pathway from serotonin, serotonin N-acetyltransferase (NAT) (Ellison et al. 1972; Hong and Pang 1995) and hydroxyindole-O-methyltransferase (Quay and Ma 1976), were found in the GIT and a daily rhythm of NAT activity was observed in the quail duodenum (Hong and Pang 1995). Higher activity of NAT was measured at midnight and lower activity at midday, suggesting the same pattern of the enzyme activity in the gut as demonstrated in the pineal gland. Since serotonin is widely distributed throughout the GIT and immunoreactive melatonin in gut was found to exhibit a regional distribution very similar to the density of enterochromaffin cells of intestinal mucosa (Bubenik et al. 1977) that are the site of 5-HT synthesis (Erspamer and Asero 1952), local production of melatonin in enterochromaffin cells was suggested (Raikhlin et al. 1975; Bubenik et al. 1977; Huether 1994).

Melatonin production in the pineal gland is driven predominantly through regulation of NAT activity (Binkley 1979; Binkley 1988) and synthesis of melatonin precursor 5-HT in the brain is controlled mainly at the level of tryptophan hydroxylation (Grahame-Smith 1971). Different situation may occur in the gastrointestinal tract where an increased local availability of melatonin precursors (e.g. in food) could influence melatonin synthesis. In order to elucidate this possibility we treated chickens with tryptophan (via gastric cannula) and subsequently measured melatonin concentrations in the pineal gland, plasma and gut.

Materials and Methods

Broiler chickens of both sexes, purchased from a local hatchery, were kept under a 12L:12D lighting regimen, with light on from 24.00 till 12.00. Light was provided by fluorescent tubes (Osram, Germany) that produced an illumination of 90 - 100 lux at the center of the room. The temperature was kept at 24 ± 2 °C. Feed (complete feed mash for broiler chickens) and water were available ad libitum. The day before experiment birds were weighed and labeled.

In the first experiment we tested effects of L-Trp administration on melatonin levels in the plasma and tissues of two-week-old broiler chickens, 60 min post load during the daytime. Tryptophan (Sigma, St. Louis, USA) was dissolved in saline and given orally as a warm solution (37 °C) by intragastric gavage (150 mg·kg^{-1} of body mass) into proventriculus of five chickens at 8.00 h. At this time 5 control birds were killed by decapitation. Treated chickens were decapitated one hour after L-Trp loading. After collection of the trunk blood in heparinised tube, the pineal gland was quickly removed and stored at -18 °C. Blood was centrifuged under refrigeration at 1500 *g* for 10 min and plasma samples were collected and stored at -18 °C. Samples of GIT (duodenum, ileum and colon) were cleaned with filter paper and stored at -18 °C.

In the second experiment we tested the influence of L-Trp loading on melatonin plasma and tissue values 3 h post load. Three-week-old broiler chickens were given L-Trp during both the light- and dark-time (6:00 h, 15:00 h, respectively 6 animals in each group) and decapitated 3 h later. Tryptophan solution was prepared and applied as described previously. Nighttime L-Trp load was performed in total darkness. Control sampling was performed in the middle of the light and dark period (6 animals in each group). Samples of the plasma and tissues were collected and stored as described previously.

Melatonin in the plasma, pineal glands and gut tissue was measured by radioimmunoassay (RIA) (Fraser et al. 1983) directly in plasma or after solvent extraction of pineal glands and gut tissue. The RIA was validated in our laboratory for using in chick plasma, pineal glands and eye tissues (Zeman et al. 1992). Sheep melatonin antiserum (G/S/704-8486), Stockgrand Ltd., Guildford, U. K.) and ^{3}H-labelled melatonin with a specific activity

Table 1
Melatonin concentrations in the pineal gland, plasma, duodenum and ileum of broiler chickens 1 h after tryptophan loading. All samples were taken during the light period. Data are presented as mean ± S. E. M. of 5 samples.

	Control	L-Trp load
Pineal gland (pg/gland)	421.30 ± 137.170	502.91 ± 159.277
Plasma (pg/ml)	18.29 ± 2.588	24.45 ± 3.587
Duodenum (pg/g of wet tissue)	52.67 ± 14.170	64.37 ± 22.890
Ileum (pg/g of wet tissue)	57.79 ± 15.980	35.95 ± 4.460

of 1.3 TBq/mmol (NEN Du Pont) were used. The activity of labelled melatonin was about 8 000 dpm and binding in Bo (without unlabelled hormone) was approximately 30 %. The parallelism of regression between the standard curve and successive dilution (n=5) of blood and extracted homogenate of gut tissue showed the absence of interferences in the assay. The sensitivity of the method was 1.5 - 2.0 pg/tube. Intra- and interassay coefficients of variation for a blood plasma pool containing melatonin at 350 pg/ml were 6.8 (n=10) and 12.1 (n=13), respectively.

Pineal glands were extracted by methanol. After homogenization, samples were dried, dissolved in 0.3 ml of tricine buffer (Sigma, St. Louis, USA) and stored at -18 °C until assay. Samples of GIT were homogenized in bidistilled water and extracted with chloroform. After centrifugation at 1500 *g* for 10 min, the aquatic phase was removed and supernatant was dried under vacuum. Subsequently the rest was washed with 1 ml of chloroform and evaporated again. The samples were dissolved in 2.5 ml of tricine buffer and stored at -18 °C until assay. The percentage of extraction was estimated on the basis of added ^{3}H melatonin (3000 dpm for sample) and represented approximately 64.2 %. Statistical analysis of data was performed using the Student's unpaired t-test.

Results

Experiment 1: Effect of tryptophan administration on daytime melatonin concentrations in the pineal gland, plasma and GIT of broiler chickens 1 hour post-load

Pineal and plasma melatonin levels (Tab. 1) were not significantly affected by L-Trp administration within 1 hour and tryptophan treatment also did not influence melatonin content either in the duodenum or ileum segment of the gastrointestinal tract.

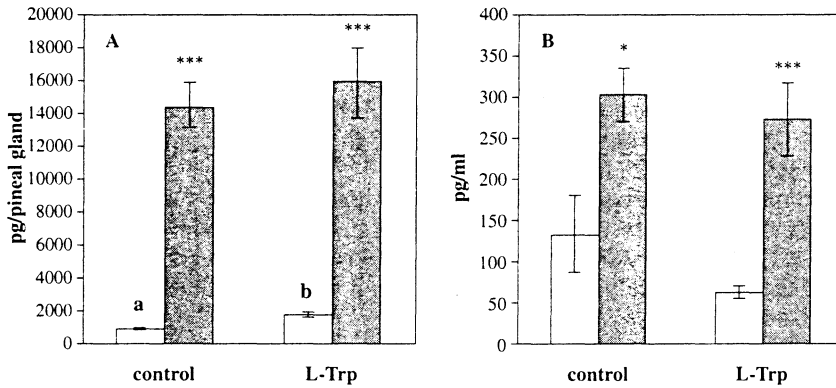

Fig. 1: Melatonin levels in the pineal gland (A) and plasma (B) of broiler chickens 3 h after tryptophan loading. Samples were taken during the light-phase (white columns) and dark-phase (grey columns). Data are presented as mean ± S. E. M. of 6 samples. Values corresponding to the same phase of the LD cycle with different letters are significantly different ($P < 0.05$). Asterisk indicate significant differences between day and night values. * $P < 0.05$, *** $P < 0.001$

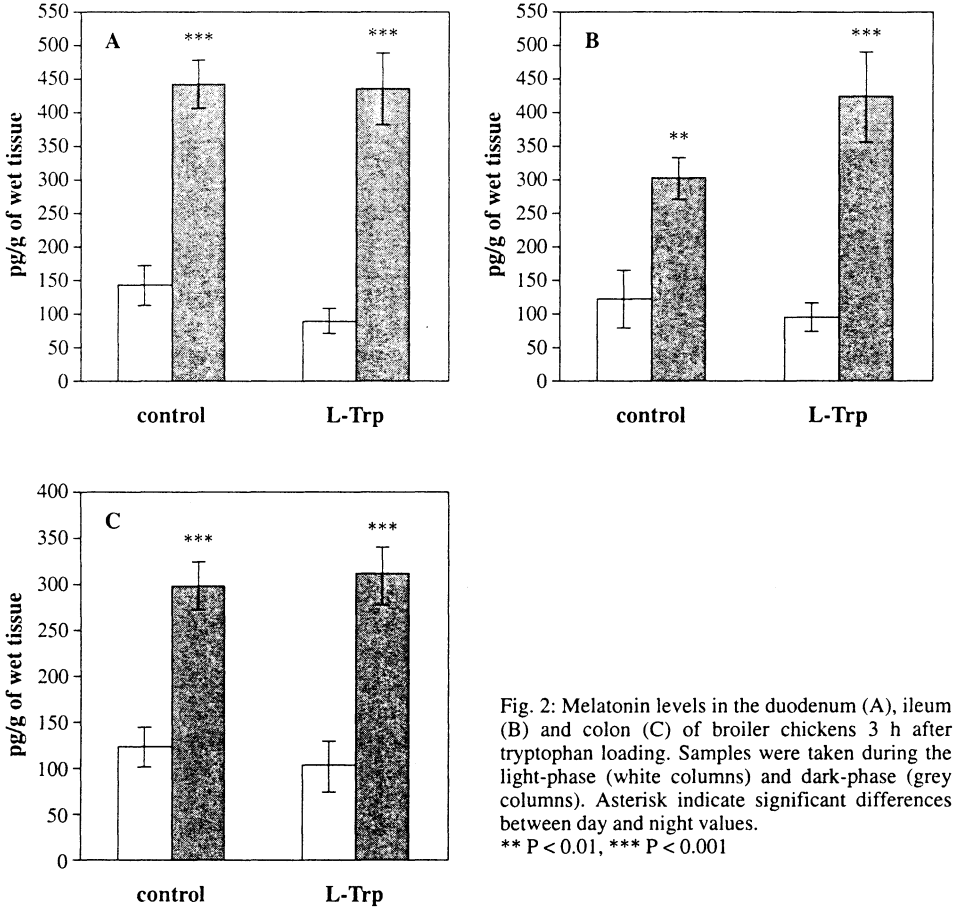

Fig. 2: Melatonin levels in the duodenum (A), ileum (B) and colon (C) of broiler chickens 3 h after tryptophan loading. Samples were taken during the light-phase (white columns) and dark-phase (grey columns). Asterisk indicate significant differences between day and night values.
** $P < 0.01$, *** $P < 0.001$

Experiment 2: Effect of tryptophan administration on daytime and nighttime melatonin concentrations in the pineal gland, plasma and GIT of broiler chickens 3 hours post-load

Tryptophan load caused a two-fold rise ($P < 0.05$) of daytime pineal melatonin content (from 795.96±110.2 to 1745.44±220.2 pg/pineal gland). In spite of this, daytime plasma levels remained unchanged. No significant difference in nighttime plasma and pineal melatonin concentrations after tryptophan treatment was found (Fig. 1).

In all parts of the GIT we observed a clear rhythm in the melatonin content with low levels during the day and high levels during the night ($P < 0.01$). Melatonin concentrations in different parts of the GIT were similar. Neither daytime nor nighttime values were affected by L-Trp administration (Fig. 2).

Discussion

Our results confirmed the presence of melatonin in the duodenum, ileum and colon of chickens. Melatonin levels in GIT exhibited a pronounced daily rhythm that paralleled that

seen in the pineal gland and plasma. These results are in accordance with data of Vakkuri et al. (1985) who demonstrated the daily melatonin rhythm in the duodenum of pigeons. The range of plasma, pineal and GIT concentrations of melatonin in our experiments are consistent with data in the literature (Vakkuri et al. 1985; Binkley 1988; Lee et al. 1995).

In our experiments we estimated influence of melatonin precursor L-Trp on melatonin levels in the GIT, pineal gland and plasma. The amount of tryptophan used in this study is similar (Huether et al. 1992) or lower (Yaga et al. 1993) as in previously published related studies and accounts for approximately daily intake of this amino acid by broiler chicken (Carew et al. 1983).

The pineal, plasma and GIT melatonin levels were not influenced by L-Trp administration 1 hour post load but three hours after L-Trp loading we observed a significant enhancement of the pineal melatonin content during the daytime. Nighttime pineal as well as both daytime and nighttime plasma and GIT melatonin levels were unaffected by L-Trp treatment. Thus our results indicate that the increased availability of L-Trp may modulate to some extent daytime melatonin levels in the pineal gland but the well known distinct melatonin rhythm in the plasma, pineal gland and GIT was not seriously affected.

Studies aimed at the influence of availability of melatonin precursors on its metabolism brought controversial results. Tryptophan depletion decreased melatonin concentration in plasma of man at night and consecutive administration of L-Trp during the day caused an increase in plasma melatonin level during the next night (Zimmermann et al. 1993). On the other hand, Hajak et al. (1991) demonstrated an enormous rise of plasma melatonin concentration after L-Trp infusion during both daytime and nighttime. Namboodiri et al. (1983) and Sugden et al. (1985) showed that administration of L-Trp did not influence daytime serum melatonin level in sheep but Yaga et al. (1993) observed elevation of plasma melatonin concentration after L-Trp loading in rat during the daytime without any effects on the pineal gland.

In the only study performed so far in chickens L-Trp administration increased considerably melatonin concentration in plasma (Huether et al. 1992). The authors suggest that this increase may be caused by L-Trp stimulation of melatonin synthesis in extrapineal site - most probably in the GIT. This suggestion is supported by findings of the above mentioned authors who noted a rise in plasma melatonin levels in both intact and pinealectomized animals after L-Trp treatment (Huether et al. 1992; Yaga et al. 1993). In our study, melatonin was measured in the gastrointestinal tract of chickens after L-Trp administration but the results do not support the prediction that the increased availability of L-Trp rises melatonin synthesis in the gut and its release into circulation.

We found higher melatonin level in the GIT than in the plasma and this finding suggests either an increased accumulation of this indole in the gut or its local synthesis in enterochromaffin cells. As rhythmic changes of melatonin levels found in the gut paralleled those observed in the pineal gland and plasma, it is evident that at least some amount of melatonin in GIT comes from the pineal gland. This conclusion is further supported by finding of Bubenik (1980) who using an immunohistochemical method demonstrated that exogenously administered melatonin is accumulated in all parts of the gastrointestinal tract of the rat. Hence, it seems probable that melatonin in GIT comes at least from two sources. The first and major one is obviously the pineal gland that imposes the daily rhythm of melatonin levels on the GIT and the second is a local one, situated probably in enterochromaffin cells of intestinal mucosa.

Taken together, we did not find remarkable differences in effect of L-Trp loading on melatonin levels in the pineal gland and GIT of chickens and additional experiments are in progress to evaluate factors influencing the local melatonin synthesis in the gastrointestinal tract of birds.

Vplyv aplikácie tryptofánu na koncentrácie melatonínu v epifýze, plazme a gastrointestinálnom trakte kurčiat

Melatonin sa podieľa na regulácii širokého spektra fyziologických procesov vrátane modulácie gastrointestinálnych funkcií. Prítomnosť vysokých koncentrácií tohto indolamínu v tráviacom trakte (GIT) bola dokázaná, ale faktory ovplyvňujúce jeho obsah v tomto systéme stále nie sú známe. Preto sme sa rozhodli ozrejmiť, či perorálne podanie prekurzoru melatonínu tryptofánu (L-Trp 150 $mg.kg^{-1}$) ovplyvní hladiny melatonínu v tráviacom trakte, epifýze a plazme brojlerových kurčiat. Koncentrácie melatonínu boli stanovené rádioimunoanalyticky po extrakcii z tkaniva. Vzorky sa odobrali pred ošetrením a 60 min (aplikácia v strede svetlej fázy) alebo 3 h (aplikácia v strede svetlej a tmavej fázy) po intubácii L-Trp. Jednu hodinu po podaní L-Trp sme nepozorovali zmeny v koncentráciách melatonínu. Tri hodiny po aplikácií L-Trp koncentrácie melatonínu v epifýze signifikantne stúpli počas svetlej fázy dňa. Nočné hladiny v epifýze, ako aj koncentrácie v plazme a GIT počas svetlej i tmavej fázy dňa zostali nezmenené. Prezentované výsledky indikujú, že zvýšená dostupnosť prekurzoru melatonínu L-Trp môže modulovať hladiny melatonínu v epifýze, plazme a čreve kurčiat iba v obmedzenom rozsahu.

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References

BARAJAS-LÓPEZ, C., PERES, A. L., ESPINOSA-LUNA, R., REYES-VáZQUEZ, C., PRIETO-GÓMEZ, B. 1996: Melatonin modulates cholinergic transmission by blocking nicotinic channels in the guinea-pig submucous plexus. Eur. J. Pharmacol. **312**: 319-325

BARANYIOVÁ, E. 1990: Effects of serotonin on the food intake in chickens in the post-hatching period. Acta. vet. Brno **59**: 23-33

BARANYIOVÁ, E. 1996: Intraperitoneal administration of tryptophan decreases liquid diet intake in early weaned piglets. Acta. vet. Brno **65**: 185-192

BINKLEY, S. 1979: Pineal rhythms in vivo and in vitro. Comp. Biochem. Physiol. **64A**: 201-206

BINKLEY, S. 1988: The pineal: Endocrine and neuroendocrine function. New York, Prentice Hall, Englewood Cliffs, 1-288 p.

BUBENIK, G. A. 1980: Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm. Res. **12**: 313-323

BUBENIK, G. A. 1986: The effect of serotonin, N-acetylserotonin and melatonin on spontaneous contractions of isolated rat intestine. J. Pineal Res. **3**: 41-54

BUBENIK, G. A., DHANVANTARI, S. 1989: Influence of serotonin and melatonin on some parameters of gastrointestinal activity. J. Pineal Res. **7**: 333-334

BUBENIK, G. A., PANG, S. F. 1994: The role of serotonin and melatonin in gastrointestinal physiology: Ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback. J. Pineal Res. **16**: 91-99

BUBENIK, G. A., BROWN, G. M., GROTA, L. J. 1977: Immunohistological localization of melatonin in the rat digestive system. Experientia. **33**: 662-663

BUBENIK, G. A., PANG, S. F., HACKER, R. R., SMITH, P. S. 1996: Melatonin concentrations in serum and tissues of porcine gastrointestinal tract and their relationship to the intake and passage of food. J. Pineal Res. **21**:251-256

CAREW, L. B., ALSTER, F. A., FOSS, D. C., SCANES, C. G. 1983: Effect of a tryptophan deficiency on thyroid gland, growth hormone and testicular functions in chickens. J. Nutrition **113**: 1756-1765

DENBOW, D. M., Van KREY, H. P., CHERRY, J. A. 1982: Feeding and drinking response of young chicks to injections of serotonin into the lateral ventricle of the brain. Poult. Sci. **61**: 150-155

ELLIOTT, J.A. 1976: Circadian rhythms and photoperiodic time-measurement in mammals. Fed. Proc. **35**: 2339-2346

ELLISON, N., WELLER, J., KLEIN, D. C. 1972: Development of a circadian rhythm in the activity of pineal serotonin N-acetyltransferase. J. Neurochem. **19**: 1335-1340

ERSPAMER, V., ASERO, B. 1952: Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature **169**: 800-801

FRASER, S., COWEN, P., FRANKLIN, M., FRANEY, C., ARENDT, J. 1983: Direct radioimmunoassay for melatonin in plasma. Clin. Chem. **29**:396-397

GRAHAME-SMITH, D. G. 1971: Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J. Neurochem. **18**: 1053-1059

GWINNER, E. 1989: Melatonin and the circadian system of birds: Model of internal resonance. In: HIROSHIGE, T., HONMA, K.: Circadian Clocks and Ecology. Sapporo, Hokkaido University Press, pp. 127-145

HAJAK, G., HUETHER, G., BLANKE, J., BLÖMER, M., FREYER, C., POEGGELER, B., REIMER, A., RODENBECK, A., SCHULZ-VARSZEGI, M., RÜTHER, E. 1991: The influence of intravenous L-tryptophan on plasma melatonin and sleep in men. Pharmacopsychiat. **24**: 17-20

HERICHOVÁ, I., ZEMAN, M. 1996: Perinatal development of melatonin production in gastrointestinal tract of domestic chicken. In: TÖNHARDT, H., LEWIN, R.: Investigations of Perinatal Development of Birds. Berlin, Free University, pp. 117-122

HONG, G. X., PANG, S. F. 1995: N-acetyltransferase activity in the quail (*Coturnix coturnix jap.*) duodenum. Comp. Biochem. Physiol. **112B**: 251-255

HUETHER, G., POEGGELER, B., REIMER, A., GEORGE, A. 1992: Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci. **51**: 945-953

HUETHER, G. 1994: Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin. Ann. NY Acad. Sci. **719**: 146-158

JANKOVIC, D., ISAKOVIC, K., PETROVIC, S. 1970: Effect of pinealectomy on immune reactions in the rat. Immunology **18**: 1-6

LEE P. N., SHIU S. Y. W., CHOW P. H., PANG S. F. 1995: Regional and diurnal studies of melatonin and melatonin binding sites in the duck gastrointestinal tract. Biol. Signals. **4**: 212-224

NAMBOODIRI, M. A., SUGDEN, D., KLEIN, D. C., MEFFORD, I. N. 1983: 5-hydroxytryptophan elevates serum melatonin. Science **221**:659-661

QUAY, W. B., MA, Y. H. 1976: Demonstration of gastrointestinal hydroxyindole-O-methyltransferase. Int. Rep. Clin. Sci., Med. Sci. Lib. Compend. **4**: 563

RAIKHLIN, N. T., KVETNOY, I. M., TOLKACHEV, V. M. 1975: Melatonin may be synthesised in enterochromaffin cells. Nature **255**: 344-345

REITER, R. J. 1991: Melatonin: the ubiquitously acting pineal hormone. News Physiol. Sci. **6**: 223-227

REITER, R. J., TANG, L., GARCIA, J. J., MUNOZ-HOYOS, A. 1997: Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci. **60**: 2255-2271

SUGDEN, D., NAMBOODIRI, M. A., KLEIN, D. C., GRADY, R. K., MEFFORD, I. N. 1985: Ovine pineal indoles: effects of L-tryptophan or L-5-hydroxytryptophan administration. J. Neurochem. **44**: 769-772

VAKKURI O., RINTAMAKI H., LEPPALUOTO J. 1985: Presence of immunoreactive melatonin in different tissues of the pigeon *(Columbia livia)*. Gen. Comp. Endocrinol. **58**: 69-75

VANT HOF, T. J., GWINNER, E. 1996: Circadian variation in Zebra finch (*Poephila gutata*) gastrointestinal tract melatonin. Ital. J. Anat. Embryol. **101**: 78

YAGA, K., REITER, R. J., RICHARDSON, B. A. 1993: Tryptophan loading increases daytime serum melatonin levels in intact and pinealectomized rats. Life Sci. **52**: 1231-1238

ZEMAN, M., GWINNER, E., SOMOGYIOVÁ, E. 1992: Development of melatonin rhythm in the pineal gland and eyes of chick embryo. Experientia **48**: 765-768

ZIMMERMANN, R. C., MCDOUGLE, C. J., SCHUMACHER, M., OLCESE, J., MASON, J. W., HENINGER, G. R., PRICE, L. H. 1993: Effects of acute tryptophan depletion on nocturnal melatonin secretion in humans. J. Clin. Endocrinol. Metab. **76**: 1160-1164