Plasma serotonin levels in Italian Fresian dairy cows

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Abstract The aim of this work was to investigate the metabolism of plasma serotonin or 5-hydroxytryptamine (5-HT), an important neurotransmitter, in Fresian dairy cows, a breed of zootechnical interest, using high-performance liquid chromatography with electrochemical detection. The subjects under study were at the stage of early lactation ($n=10$; mean body weight 375 ± 50 kg; average age of 3 years; body condition score 2.5), bred in a farm at an altitude of 150 m a.s.l. To evaluate animal welfare on this farm, which is closely connected to an animal's physiological status, tryptophan and cortisol levels (measured by enzyme-linked immunosorbent assay), together with levels of certain blood components [total proteins (TP), albumin, creatinine, glucose (Glu), triglycerides, phospholipids, total cholesterol, and aspartate transaminase, measured by spectrophotometry] were analyzed. The results obtained are discussed in comparison with reference values, taking into account the environmental living conditions. Measured plasma serotonin concentrations, which were lower than values reported for Brown Swiss dairy cows of a comparable age and diet, appeared to be affected by breed, temperature, blood sampling season, and altitude. Additional differences between the levels of plasma tryptophan, the amino acid precursor of serotonin, of the two breeds were comparable. Negative correlations between plasma tryptophan and plasma cortisol levels ($r=-0.83$, $P<0.005$), plasma serotonin and plasma TP levels ($r=-0.72$, $P<0.05$), or Glu levels ($r=-0.77$, $P<0.05$) highlight the existence of a stress condition, which is connected to an energetic deficit related to lactation.

Keywords Fresian dairy cows · Plasma serotonin · Biochemical parameters · Tryptophan

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Abbreviations

5-HT Serotonin

MAO monoamino oxidase

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter of the central and peripheral nervous system and is involved in many important biological functions, still incompletely understood. 5-HT acts as a vasoconstrictor on lung smooth muscles, the cardiovascular system, and cerebral arteries. It is synthesized from the amino acid tryptophan (Try) in the brain, mastocytes, and enterochromaffin cells of the gut. Ninety-seven percent of the total body amount of 5-HT is held in platelets (Andres et al. 1993), which do not synthesize it, but function as the main transporter into peripheral blood. In mammary glands, serotonin plays a role as a regulator of the short-term homeostasis of milk yield (Stull et al. 2007). In fact, mammary glands stimulated by prolactin (PRL) expressed essential genes for serotonin biosynthesis. Tryptophan hydroxylase mRNA increased during pregnancy and lactation with consequent 5-HT found in mammary gland epithelial cells and in milk (Matsuda et al. 2004). Plasma 5-HT, which has a rapid turnover distinct from that of platelet 5-HT, is the fraction which shows the equilibrium state between synthesis by enterochromaffin cells, the inactivation by liver and lung monoamino oxidase (MAO), and platelet uptake. Its role is still unclear. 5-HT has been deeply investigated only in humans during physiological (Hirvonen et al. 2002) and pathological conditions (Lee et al. 2000). Plasma 5-HT levels of Brown Swiss dairy cows measured by ELISA were previously reported (Kollmann et al. 2008). The aim of this work was to further investigate the metabolism of plasma serotonin (5-HT) in a breed of zootechnical interest, the Italian Fresian, which is used for milk production. Through the use of a more sensible methodology, HPLC with coulometric detection, together with the analysis of some blood and hormonal parameters, we were able to underline the animal welfare and/or existence of some possible stress conditions that may impair reproductive performance and milk production.

Materials and methods

The research was carried out on N.10 Italian Fresian dairy cows during early lactation (mean body weight 375 ± 50 kg; average age of 3 years; BCS 2.5). Cows were bred on a farm in Messina at an altitude of 150 m above sea level, were freely stabled, and were fed a mixed ration of concentrates (50%) and fresh forages (50%). Blood samples were collected into EDTA-containing collection vessels from the jugular vein at 9:00 a.m. after conventional milking at temperatures of 10–15°C in February. Following haematocrit (HCT %) measurements, aliquots were centrifuged at $2000 \times g$ and the following plasma parameters were evaluated: total proteins (TP), albumin (ALB), creatinine (Crea), glucose (Glu), triglycerides (TG), phospholipids (PLP), total cholesterol (TCho), and aspartate transaminase (AST) by spectrophotometry. Cortisol levels, in duplicate, were measured by ELISA (Radim). The remaining fraction was centrifuged at $4500 \times g$ to obtain platelet poor plasma (PPP). The same volumes of N-methylserotonin (internal standard) and protein precipitation reagent (Chromsystems) were added to 100 μ l of each sample. The solutions were vortex-mixed, incubated at 4°C, and centrifuged at 13000 rpm. The supernatant was used for the detection of plasma 5-HT and tryptophan (Try) by reverse phase HPLC (Waters

Table 1 Blood parameters (Mean±S.D.) in Fresian dairy cows

HCT (%)	Glu (mg/dl)	PLP (mg/dl)	TCho (mg/dl)	TG (mg/dl)	AST (U/l)	Crea (mg/dl)	TP (g/dl)	Alb (g/dl)
22±2.1	63±4.6	173±30	120±30	61±26	66±9	1.27±0.22	6.61±0.41	2.54±0.18

1525 binary HPLC pump) with an electrochemical detector (ESA) and an experimental time of 16 min. Statistical analyses used the Student's paired *t*-test and correlation and linear regression tests.

Results and discussion

Blood parameters (Table 1) were comparable with those previously reported for the same breed (Giulioti et al. 2004) and highlight a condition of good health, good fitting to the environment, and an absence of peculiar clinical signs, therefore suggesting a general state of welfare. Albumin levels in some subjects were slightly lower. Triglyceride concentrations were higher in all subjects and were probably related to diet. Plasma serotonin, tryptophan, and cortisol levels were measured in Fresian dairy cows and were compared with previous data from Brown Swiss dairy cows (Kollmann et al. 2008) (Table 2).

The subjects of both breeds had a comparable average age and were fed an isoenergetic diet. The differences between the 5-HT values that were obtained by different methodologies were probably related to factors such as breed, season of sample taking, specifically winter time for Fresian and summer time for Brown Swiss, and eventually differences in altitude. In both human and animals, altitude may affect hypoxia conditions with consequential pulmonary hypertension. Among mammals, the first clinical signs of hypoxic pulmonary hypertension came from investigations carried out on bovines, which then became the first animal model for the study of this pathology (Rhodes 2005). Moreover, experiments carried out on hypoxic rats highlighted a decrease in serotonin uptake in platelets (Awabdy et al. 2003). At higher altitudes, platelet function could be underregulated resulting in higher plasma serotonin levels. Also differences between the two breeds of plasma tryptophan, the amino acid precursor of serotonin, were comparable and followed the same trend. The negative correlation between plasma tryptophan and cortisol ($r=-0.83$, $P<0.005$) highlights, as we already know in humans (Ventura and Zeneroli 1982), the presence in bovines of an opposite trend of these two parameters. Cortisol levels remarkably higher than normal values (Medica et al. 2007) point out the presence of a physiological stress condition connected to an energetic deficit related to lactation. This hypothesis is confirmed by the BCS

Table 2 Plasma serotonin (5-HT), tryptophan (Try), and cortisol levels (Mean±S.D.) in dairy cows

BREED	5-HT (ng/ml)	Try (μg/ml)	Cortisol (ng/ml)
Fresian	170±50	3.73±1.23	59.5±16.4
Brown Swiss ^a	334±81	5.72±0.60	

^aKollmann et al. 2008

values, the presence of significant negative correlations between tryptophan and cortisol levels ($r=-0.83$, $P<0.005$), 5-HT and TP ($r=-0.72$, $P<0.05$), and 5-HT and glucose ($r=-0.77$, $P<0.05$), and at the limit of significance between 5-HT and HCT ($r=-0.69$, $P=0.05$). The subject of this study will be further investigated with the same breed and additional parameters related to energetic and mineral status (NEFA, β -HB, Ca, P) will be analyzed in a greater number of subjects and during different lactation stages.

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