Short Communication

Effect of age and blood collection site on the metabolic profile of ostriches

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Abstract

The serum metabolic profile of ostriches was studied in relation to the blood collection site (jugular *vs.* wing vein) and age (1 *vs.* 2 years) on 20 male birds. Blood was collected from the birds in the morning, after 12 h of fasting. Different collection site did not affect the examined parameters, but some statistically significant differences were observed between the age groups. However, all the parameters agreed with the data reported in the literature and contribute to our knowledge of the metabolic profile of ostriches.

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Blood profiling, initially used to detect subclinical metabolic disorders due to incorrect feeding, has recently been used more widely to evaluate the effects of different treatments on metabolic, nutritional and welfare conditions of animals (Bertoni *et al*., 2000). In the past, bovines were studied extensively, but now, given the interest of the scientific community, metabolic profiles are studied in all livestock species. In recent years ostriches (*Struthio camelus domesticus*) have been increasingly farmed in Europe, with more than 40 000 head in Italy alone (ISTAT, 2000). In spite of this, there is relatively little knowledge on the blood serum profile of ostriches (Van Heerden *et al*., 1985; Levy *et al*., 1989; Angel, 1996; Brown & Jones, 1996). Given that it is necessary to have standard values and a knowledge of their variation in relation to age, season, physiological status, blood collection methods, stress and other factors for proper evaluation of metabolic profiles, the aim of this study was to evaluate the effect of the blood collection site and of age on the metabolic profile in ostriches.

The research was carried out on 20 male ostriches equally divided into two age groups, 12 *vs*. 24 months. The birds were hatched and reared on a commercial farm on Sardinia (Italy). The ostriches were fed *ad libitum* on a diet consisting, on a dry matter basis, of 25% maize silage, 50% lucerne hay and 25% of a commercial concentrate. The chemical composition of the diet, determined according to procedures of AOAC (1984) and Van Soest *et al*. (1991), is reported in Table 1.

Table 1 Chemical composition of diet (g/kg DM)

Crude protein	177.1
Ether extract	26.0
Crude fibre	237.3
Ash	78.2
Neutral detergent fibre	342.1
Acid detergent fibre	236.4
Lignin	59.0

In the morning (from 08:00 to 10:00), following about 12 h fasting, blood was collected from each ostrich by vacutainer from both the jugular vein and the left subcutaneous ulnae wing (v. *ulnaris subcutanea*) vein. Within one hour, the blood samples were centrifuged in order to obtain the serum which was immediately frozen at -21 °C until analysis. The latter was carried out in the Animal Biology Department of

the University of Sassari (Italy), using an automated system (Ektachem 250 analyzer, KODAK). Each sample was dispensed on a multilayer support in the middle of which there was one which was absorbed with reagents. These led to the formation of a final coloured compound, which was quantified by a spectrophotometer and from which the concentration of the metabolite was obtained. The analyses were made using the following methods:

- colorimetric for calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), glucose, total protein (TP), cholesterol, triglycerides, lactate (LAC), uric acid, total bilirubin (Tbil) and γ-g1utamy1transferase (GGT);
- enzymatic-colorimetric for creatinine (CREA), α-amylase (Amyl), lipase (LIP);
- spectrophotometric for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and cholinesterase (ChE);
- potentiometric for sodium (Na), potassium (K) and chloride (Cl), by specific electrodes.

Amyl values were converted in their natural logarithms in order to normalize the distribution. Statistical analyses of the results were carried out by ANOVA (SAS, 2000), using the model:

$$Y_{ijk} = \mu + A_i + S_j + \varepsilon_{ijk}$$

where:

Y = the single observation;
μ = the general mean;
A = the effect of age (i = 12 or 24 months);
S = the effect of collecting site (j = jugular *vs.* wing vein);
ε = the error.

As it was not statistically significant, the interaction between the two considered effects was not included in the model.

From our experience, blood collection from the wing vein was easier than from the jugular vein. In both cases, the ostriches were hooded with a dark cap and restrained in a corner in order to contain their movements but when the blood was collected from the neck, the animals reacted to the pressing, moving their heads, while, when the blood was collected from the wing vein the animals were calmer and the collection easier and faster.

All ostriches were healthy when the blood was collected and did not show any clinical signs of any disease within one month before or after sampling. The results of the serum analyses by collection site and age are reported in Table 2. The collection site did not affect the metabolic profiles, but some biochemical parameters changed with age.

The concentrations of the main parameters associated with energy metabolism (glucose, cholesterol and triglycerides) did not differ between the two age groups (Table 2) and were in agreement with those reported in the literature (Van Heerden *et al*., 1985; Levy *et al*., 1989). Costa *et al*. (1993) reported for the emu (*Dromaius novaehollandiae*), a species closely related to the ostrich, a low glucose concentration from the first to eight weeks of age, but very similar values between the eighth week and maturity. Since all ostriches in the present study were over one year old, such age differences were not evident. However, the ostriches showed lower glucose and cholesterol concentrations and similar triglycerides values than other poultry (Franchini *et al*., 1990; Chiericato & Rizzi, 1999a; b).

The protein concentrations in blood serum showed similar values at the two considered ages (Table 2). These results are in agreement with those (37 - 39 g/L) reported by Levy *et al*. (1989), Palomeque *et al*. (1991) and Okotie-Eboh *et al*. (1992), but slightly lower than those (41 - 45 g/L) recorded by Van Heerden *et al*. (1985) and Angel (1996). In all cases, the serum protein concentrations of ostriches were in agreement with an average value of 40 g/L, reported for birds (Bell & Freeman, 1971). Uric acid and creatinine concentrations are also in agreement with other findings (Levy *et al*. 1989). Creatinine concentrations increase when muscular tissue turnover accelerates (Finco, 1989). Higher values of blood creatinine were observed by Campanile *et al*. (1990) in lambs treated with clenbuterol, which is associated with an increase in muscle mass. In this regard Fekry *et al*. (1989) asserted that modifications in blood creatinine concentration could be useful to estimate changes in protein mass in muscles. In fact, excluding kidney damage, an intense turnover of muscle proteins induces an immediate increase in blood creatinine concentration (Finco, 1989), while a low mobilisation of the same proteins decreases the level of creatinine

in the blood. In the present study, the creatinine concentrations were not statistically different between the two age groups (Table 2). It could therefore be assumed that in growing ostriches changes in muscle mass have reduced already at one year of age.

Table 2 Metabolic profile in serum of ostriches (mean ± standard deviation) in relation to age and site of collection

Parameters		Age of ostriches*		Collection site	
		1 year	2 years	Jugular vein	Wing vein
Glucose	Mmol/L	12.40 ± 1.03	11.28 ± 1.40	11.66 ± 0.83	12.02 ± 0.76
Cholesterol	"	1.82 ± 0.53	1.75 ± 0.36	1.73 ± 0.43	1.84 ± 0.39
Triglycerides	"	1.03 ± 0.43	1.09 ± 0.45	1.03 ± 0.47	1.09 ± 0.45
LAC	"	4.65^{B} ± 0.75	6.44^{A} ± 0.96	5.27 ± 0.71	5.82 ± 0.56
TP	g/L	38.75 ± 6.75	37.36 ± 4.76	38.09 ± 4.16	38.01 ± 3.94
Uric acid	Mmol/L	381.2 ± 76.6	435.4 ± 69.4	399.9 ± 43.4	416.7 ± 39.8
Crea	"	20.35 ± 3.26	23.79 ± 7.36	20.87 ± 3.28	23.26 ± 4.02
Sodium	"	154.7^{a} ± 11.5	140.9^{b} ± 10.7	147.2 ± 6.2	148.5 ± 5.6
Potassium	"	2.78^{B} ± 0.82	3.87^{A} ± 0.40	3.39 ± 0.37	3.27 ± 0.54
Chloride	"	119.9^{A} ± .25	106.0^{B} ± 7.51	112.3 ± 4.49	113.6 ± 5.35
Calcium	"	2.50^{b} ± 0.24	2.69^{a} ± 0.15	2.55 ± 0.21	2.64 ± 0.19
Phosphorus	"	1.72 ± 0.53	1.92 ± 0.35	1.81 ± 0.16	1.84 ± 0.20
Magnesium	"	0.87 ± 0.13	0.84 ± 0.17	0.84 ± 0.15	0.86 ± 0.18
Iron	"	5.48^{B} ± 1.64	8.53^{A} ± 1.52	7.33 ± 1.73	6.69 ± 1.44
Tbil	"	6.80^{b} ± 0.93	9.36^{a} ± 1.49	7.84 ± 1.32	8.32 ± 1.01
AST	U/L	384.6^{A} ± 3.3	249.6^{B} ± 54.6	323.2 ± 35.4	311.1 ± 29.1
ALT	"	14.35^{A} ± .45	9.93^{B} ± 2.40	12.75 ± 1.97	11.53 ± 2.48
AP	"	49.00^{B} ± .15	70.82^{A} ± 5.09	58.34 ± 4.11	61.48 ± 4.36
GGT	"	12.75^{A} ± .34	7.66^{B} ± 1.11	9.74 ± 2.27	10.66 ± 1.75
Amyl	"	7.07^{A} ± 0.64	6.85^{B} ± 0.54	6.99 ± 0.23	6.93 ± 0.39
LIPA	"	322.1^{a} ± 54.3	237.8^{b} ± 91.3	279.3 ± 43.21	280.6 ± 5.98
ChE	"	653.1^{A} ± 3.4	496.0^{B} ± 42.5	565.1 ± 39.67	584.0 ± 7.43

* Differences in rows within age groups with superscripts A, B are significant at $P < 0.01$, and a, b at $P < 0.05$
LAC – lactate; TP - total protein; Tbil - total bilirubin; Crea - creatinine; AST – aspartate aminotranferase; ALT – alanine aminotranferase; AP - alkaline phosphatase; GGT – γ-g1utamy1transferase; Amyl - amylase; LIPA - lipase; ChE – cholinesterase

The activity of the serum enzymes (GGT, AST, ALT, AP, LAC, Amyl, LIPA, ChE) showed significant differences depending on age. We suggest that these differences were due to the biochemical changes of different organ systems whose enzyme activities increase with age up to body maturity, at about 14 months of age (Cilliers *et al*., 1995). Obviously, tissue development and modification during the growing period could induce high serum enzyme activity. On the other hand, the lower bilirubin level in one-year old ostriches compared with the two-year old birds, in association with a similar total protein value within the physiological range of the species (Van Heerden *et al*., 1985; Levy *et al*., 1989; Angel, 1996) allows us to exclude hepatic or muscular pathologies. The GGT showed higher values than those reported in the literature (Van Heerden *et al*., 1985; Levy *et al*., 1989; Okotie-Eboh *et al*., 1992). Importantly, the analytical evaluation of enzymes is less standardised than those of other blood parameters, which may account for differences between laboratories. Moreover, regarding GGT, standard values have not yet been defined in ostriches and those recorded by Franchini *et al*. (1990) for other poultry are higher (3-9 U/L) than our findings. Alkaline phosphatase activity showed lower values than those (150-575 U/L) reported in the literature (Levy *et al*., 1989; Okotie-Eboh *et al*., 1992). This enzyme is normally higher in young animals when the bone metabolism is intense than in older animals. Accordingly, in emus Costa *et al*. (1993) reported lower AP activity in adults than in two-month old birds. In the present study, the highest values

recorded in the two-year old ostriches could be explained partly because, in one-year-olds, body maturity has nearly been reached and partly because two-year-olds are approaching sexual maturity. However, this would require experimental verification. Serum AST and ALT activity showed high variability, in correspondence with the literature, where AST values ranged from 131 U/L (Levy *et al*., 1989) to 372.2 U/L (Angel, 1996), and ALT from 2.0 U/L (Levy *et al*., 1989) to 20.62 U/L (Palomeque *et al*., 1991). Comparing the activity of liver enzymes to the results of Quintavalla *et al*. (2001) AST showed higher activity (384.5 and 249.6 U/L, for one- and two-year old ostriches, respectively), than the 164.03 and 140.06 U/L quoted by Quintavalla *et al*. (2001), but the age trends of both AST and ALT, *viz.* that one-year old birds showed the highest values, were the same.

The more intense basal metabolism of one-year old animals could explain their higher serum activity of amylase and lipase than the two-year old birds. In fact, higher thyroid hormone levels in younger birds increase cell membrane permeability, allowing an escape of intracellular amylase and lipase (Loeb & Quimby, 1989). Lower serum Ca and K concentrations seem to confirm this hypothesis because, when these enzyme activities increased, Ca and K concentrations decreased (Bertoni *et al*., 2000).

The higher levels of lactate in two-year old ostriches are probably due to more intense muscular activity of animals during capture. These birds were located in larger paddocks used for reproduction and consequently had more area available for evading capture. In contrast, the one-year old ostriches were located in a small space used for slaughter and could be captured with minimal activity on their part. Regarding ChE, one-year old ostriches had significantly higher blood concentrations than two-year-olds, which is consistent with results of Okotie-Eboh *et al*. (1992), who found that ChE concentrations decreased with age.

The blood concentration of minerals and their ratios can indicate not only subclinical pathology but especially mineral deficiencies and imbalances. As with other animals, knowledge of baseline values of these is useful in ostriches as breeders very often prepare rations neglecting the nutritive requirements of the animals. The mean blood concentrations of Ca (2.6 mmol/L), P (1.82 mmol/L) and Mg (0.85 mmol/L) are in agreement with the findings of other authors (Van Heerden *et al*., 1985; Levy *et al*., 1989; Bezuidenhout *et al*., 1994). However, it emerged that older ostriches had a higher ($P < 0.05$) blood Ca concentration than the younger ones. In emus, Costa *et al*. (1993) reported higher Ca concentrations in adults than in two-month old birds (2.87 *vs*. 2.59 mmol/L). The slight variability of Mg, given that its blood concentration is also related to its bioavailability in feed (Bertoni *et al*., 2000), is very important. The serum levels of Mg are a good indicator of Mg intake, at least in the short term. The levels of Na, Cl and K showed significant differences between the considered ages. Sodium (154.7 and 140.9 mmol/L) and Cl (119.9 and 106.0 mmol/L) showed higher values in one-year old ostriches, whereas K was higher in two-year-olds (3.87 *vs*. 2.78 mmol/L). As feedstuff is rich in K, it is very unlikely to find low blood values of this mineral unless the animals are fed a large quantity of by-products or fed cooking remainders, which could be K deficient (Bertoni *et al*., 2000). In other species, blood K levels can be lowered by stressful conditions (Bertoni *et al*., 2000). In all cases, the recorded concentrations of Na, Mg and K fell within the physiological ranges of the species (Van Heerden *et al*., 1985; Levy *et al*., 1989; Brown & Jones, 1996).

The serum Fe concentration in the one-year old birds was lower than in the two-year old ostriches (5.48 *vs*. 8.53 µmol/L, respectively). However, the lack of available data in the literature, the presence of factors that interfere with duodenal absorption of Fe (decreasing in the presence of phosphate) and the high daily variations due to the feed intake did not allow us to formulate any concrete hypothesis without the acquisition of more data.

Since no statistically significant difference was found between the two collection sites, the authors suggest that for ease of collection, blood samples from ostriches should be taken from the wing vein rather than the jugular vein. As regards the effect of the two different ages, the significant variations of some metabolic parameters fully reflected different intensities of the basal metabolism. Finally, the values found for each parameter are in broad agreement with those reported in the literature, and contribute by enhancing our knowledge, albeit still rather inadequate, in ostriches, of the variation and range of blood metabolites. They would be very useful in detecting not only metabolic-nutritional disorders but also animal welfare conditions.

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