BIOCHEMICAL STUDIES ON BIRD FLIGHT

Thesis submitted to the University of Calicut
for the Degree of Doctor of Philosophy
under the Faculty of Science

By

T.V. SHAJITHA

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, INDIA

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CERTIFICATE

This is to certify that this thesis entitled "BIOCHEMICAL STUDIES ON BIRD FLIGHT" is a bonafide research work done by T.V. Shajitha from February 2002 to July 2005 in the laboratory of Insect Physiology and Biochemistry of the department under my supervision and guidance, in partial fulfilment of the requirement of the Degree of Doctor of Philosophy under the Faculty of Science, University of Calicut. I also certify that no part of this thesis has been presented before for any other degree.

Dr. K.V. Lazar

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DECLARATION

I, T.V. Shajitha, do hereby declare that this thesis entitled "BIOCHEMICAL STUDIES ON BIRD FLIGHT" submitted by me to the University of Calicut for the award of the degree of Doctor of Philosophy under the Faculty of Science is the result of the research work carried out by me under the guidance of Dr. K.V. Lazar, Lecturer, Department of Zoology, University of Calicut. I further declare that no part of this thesis has been presented before for any other degree.

Place: Calicut University
Date : 29.07.2005.

T.V. Shajitha

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Introduction

INTRODUCTION

Avian flight has fascinated man from ancient times but it is only in recent years that the efforts of scientists from diverse field have been able, to some extent, to understand and explain the dynamics of animal flight. Birds fly over vast expanses of land and sea to reach their destination. For example, the Golden plover flies over the Atlantic Ocean covering a distance of nearly 2400 miles without stopping for food or rest and then proceeds southward to its destination in Argentina, over a land for more than 2000 miles.

Origin of avian flight is often equated with the phylogeny, ecology and flying ability of the primitive Jurassic bird, Archaeopteryx. Debate persists about whether it was a terrestrial cursor or tree dweller. Despite broad acceptance of its arboreal life style from anatomical, phylogenetic and ecological evidence, a new version of the cursorial model was proposed recently asserting that a running Archaeopteryx could take off from the ground using thrust and sustain flight in the air. However, Archaeopteryx lacked both the powerful flight muscles and complex wing movements necessary for ground take off (Chatterjee and Templin, 2003).

The specialized skeletal system of the bird provides a light framework of stability and strength. The appendicular parts of the skeleton are especially adapted to act as spring boards for muscular action. Of these two major flight muscles play the important role in elevating and depressing the humerous and thus the wing as a whole. They are pectoralis major and supracoracoideus (pectoralis minor). Both these muscles arise from the ventral surface of the sternum, thus, the centre of gravity remains low (George and Berger, 1966).

Studies on the energy metabolism of bird flight showed that their metabolism is similar to that of other animals with the exception of specialized pathways suited to its habitat. As in other animals carbohydrate appears to be a major immediate source for avian cells. However extensive fatty acid utilization and gluconeogenesis are other alternative energy sources. Tucker (1973) has suggested that a considerable portion of carbohydrate absorbed by the avian intestine is converted to fat prior to its use as an energy source. However protein is also catabolised during flight (Bauchinger and Biebach, 1998; Jenni and Jenni- Eiermann, 1998) and some of it is stored before flight bouts (Gauthier *et al.*, 1992; Lindstrom and Piersma, 1993; Marsh, 1984; Mc Landress and Raveling, 1981; Piersma and Jukema, 1990). Lipids stored in adipose tissue are the main fuel during long flights (Jenni-Eiermann *et al.*, 2002; Landys *et al.*, 2005). The present investigation concerns with the flight metabolism of birds with special emphasis on the energy resources during flight using homing pigeon as a model.

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REVIEW OF LITERATURE

Flight

In the animal kingdom, only birds, insects and bats fly with their own muscle power. Flight enables them to disperse rapidly and widely and seek new areas to colonize. It also enables them to seek new and sparsely distributed food resources, locate potential mates and search for oviposition sites (Nation, 2002).

The development of flight is seen only in four animal taxa, among all the animal groups that have evolved: that is insects, the now extinct pterosaurs, birds and bats (Maina, 2000).

Flight is the main characteristic of the birds. Almost every aspect of bird biology has been modified for an aerial existence, and bird behavior cannot be considered without reference to the benefits and problems of flight. Flight has conferred great advantages on the birds in terms of fast and sustained locomotion. Not only can they cover a large area in a day's foraging, but they can also migrate thousands of kilometers to exploit two different habitats in the course of a year. On the other hand, the changes to the body needed for flight have caused constraints on other activities. The transformation of the forelimbs into wings is so complete that they can have few other functions. Some birds use their wings for signaling in courtship displays, a few herons employ them as sunshades when fishing and young hoatzins and rails use them for clambering through branches. But, whereas mammals use their forelimbs for holding, manipulating and carrying, birds must manage with the horny bill, assisted occasionally by the feet (Burton, 1985).

Bird flight

Birds fly in a variety of ways, ranging from gliding to soaring to flapping flight to hovering. Of these, the simplest type of flight is gliding. A gliding bird uses its weight (mass) to overcome air resistance to its forward motion. To do this effectively it requires a certain mass and, as a result, only large birds, such as vultures, glide on a regular basis. When gliding, a bird loses altitude at some 'sinking speed' (Vs) while traveling forward at some 'flight speed' (V). A bird's glide ratio equals V/Vs (the distance traveled forward per unit of altitude lost). Some of the best 'bird gliders' (such as Black Vultures) may travel up to 20 meters for every meter of altitude lost (or, a glide ratio of 20).

A soaring bird (e.g., Turkey, Vultures) maintains or increases its altitude without flapping its wings. One way to do this is to take advantage of rising air. Updrafts are generated when a steady wind strikes a hill, cliff, or building, and this is referred to as obstruction lift. Thermals, or updrafts caused by the uneven heating of air near the earth's surface. Air over fields heats faster than air over a forest or lake. The warmer air over a field is lighter than the surrounding cooler air and, therefore, rises. However, at high altitudes the warm air begins to cool and sink. As a result, birds using thermals for lift typically fly in circles (to stay in the area of rising air). Over the open ocean, large birds like the Wandering Albatross take advantage of wind velocity gradients in a type of soaring called dynamic soaring.

Most birds flap their wings when they fly. Flapping flight involves up-and-down movement of the wings and, during such flight, different parts of a wing have different functions. During the downstroke (power stroke), a wing moves downward and forward. As a result, the trailing edge of the wing bends upward (due to the air pressure) and this transforms the wing into a 'propeller' and moves the bird forward. During the upstroke (recovery stroke), the tips of the primaries separate and these 'slots' allow passage of air through them (which reduces friction as the wing comes up). Also, the wing is partially folded at the wrist and elbow and drawn in toward the body to reduce drag.

Most species of birds do not flap their wings continuously during flight. Rather, they exhibit one of two intermittent flight patterns: flap-gliding and flap-bounding. Mathematical models predict that flap-bounding is energetically cheaper than continuous flapping flight at high speeds, while flap-gliding is more efficient than continuous flapping at low speeds. However, few species of bird exhibit both types of intermittent flight, so flap-bounding may be a compromise between the need to maintain muscle contractions at an optimal velocity and the need to vary power output and flight speed.

A few birds use hovering flight. Some birds, like American Kestrels, 'hover' or remain in place by flying into the wind at a speed equal to that of the wind, and other birds hover momentarily while foraging. But hummingbirds are able to remain in the same place in still air as long as they wish they are true hoverers. A hovering hummer keeps its body at about a 45 degree angle to the ground and moves its wings in more or less a figure-eight pattern, with the "eight" lying on its side. Hummingbirds, unlike other birds, can also fly backwards.

Insect flight and flight metabolism

Insects are the only invertebrate that can fly. In some insects, such as blowflies and some Hymenoptera, flight is the most energy intensive biological process known per unit weight of tissue. Perhaps because of the unique position it holds, flight metabolism has been studied extensively (see reviews, Bailey, 1975; Beenakers *et al.*, 1986; Candy, 1985; Downer, 1985; Friedman, 1985; Hainsworth, 1981; Pearson, 1950; Sacktor, 1974).

The most intense and rapid energy demands made by insects come with flight. Within seconds, the available high energy phosphates in the body are used in flight, and energy must be made available rapidly and for long periods for flight to continue. The tracheal system of insects is able to supply O_2 to mitochondria even during flight, and insects do not in an oxygen debt in flight. As a consequence of the efficiency of the tracheal system, and a fast glycerol-3-phosphate shuttle that regenerates NAD^+ for use in glycolysis, virtually all metabolic glucose can go directly to pyruvate, and pyruvate can go directly into mitochondria for metabolism to release large amounts of energy. Flight muscles mitochondria are highly specialized to support a high rate of metabolism. They are extraordinarily large and irregular in shape. The cristae of flight muscle mitochondria are numerous, like the pages of a book and there is relatively little open space within flight muscle mitochondria (Suarez *et al.*, 2000).

Some groups (Lepidoptera, Orthoptera, and some others) burn lipids (fatty acids) as flight fuels. Fatty acids, which must be metabolized within the mitochondria and hence require availability of oxygen, release large amounts of energy per unit weight of substrate metabolized. The ability to rapidly mobilize and transport lipid from fat body, and the availability of oxygen from the tracheal system, are major adaptations in those insects that burn fatty acids for flight. Insects that metabolize lipids are able to fly continuously for hours and undertake long distance migration. It has been found that the energy liberated on the oxidation of proline is used as an energy source during insect flight (Weis-Fogh, 1952).

Bat flight and flight metabolism

Bats are one of the few orders of mammals that echolocate and the only group with the capacity for powered flight. The order is sub divided into Microchiroptera and Megachiroptera, with an array of characteristics defining each group, including complex laryngeal echolocation systems in microbats and enhanced visual acuity in megabats (Teeling *et al.*, 2000).

In mammals, the duration of a muscle contraction is adapted to its function, and the contraction performance of the muscle is affected by the resistance that it works against (Guyton and Hall, 1996). The highest metabolic rates, recorded from flying bats are essentially the same as those predicted for flying birds of the same body masses, but are from 2.5 to 3.0 times greater than the highest metabolic rates of which similar size exercising terrestrial mammals appear capable (Thomas, 1975). Basel rates of metabolism within the insectivorous genera old world leaf nosed bats, ranged from 58% to 77% of the mammalian standard (Bonaccorso and Mc Nab, 2003).

Flapping flight is one of the most expensive activities in terms of metabolic cost and this cost has previously been considered equal for the two extent vertebrate groups which evolved flapping flight. Current estimates of flight cost within the group of small birds and bats may differ. Metabolic flight power of small bats was found to increase with body mass. This is about 20-25% below the majority of current predictions of metabolic flight cost for small birds. Thus, either the flight cost of small birds is significantly lower than has previously been thought or, contrary to current opinion, small bats require less energy to fly than birds (Winter and Von Helversen, 1998). Bat flight and flight metabolism has been studied extensively by many scientists (Brunnet- Rossinni *et al.*, 2004; Bullen and Mc Kenzie, 2002; Ibanez *et al.*, 2001; Kim *et al.*, 2000; Maina and King, 1984; Voigt and Winter, 1999)

Theories of the evolution of bird flight

The origin of bird flight is a fundamental issue in avian biology and in overall evolutionary theory. Many scientists point to the fossilized specimens of Archaeopteryx as evidence that there was a transitional vertebrate species that developed during the evolution from reptilian dinosaurs to birds.

Until now, there have been two basic theories on the origins of bird flight. The first, the arboreal theory (Boker, 1927) is a tree-to-ground model, suggesting that birds' primitive ancestors were tree-dwellers that leapt from branch to branch. Through the ages, the ability to first glide and later flight developed because gliding resulted in their fall to earth when they missed a branch.

The second, the cursorial theory (Nopsea, 1907) is a ground-up model that suggests that birds evolved from four-legged reptiles. According to this theory, scales on these creatures' front limbs gradually developed into feathers that gave them upward thrust when they ran and eventually enabled them to fly.

The third and new theory of evolution of bird flight is linked to parental care (Carey, 2002). The parental-care theory is consistent with both the physical and behavioral changes that appear to have occurred as reptiles evolved into winged dinosaurs and finally into modern birds.

It was suggested that modern bird's very early ancestors were reptiles that established and guarded their nests on the ground, much like crocodiles. Over time, these creatures developed hard-shelled rather than leathery eggs and the ability to modulate their own body temperature in order to provide a more constant environment for their developing young. Scales evolved into feathers, better camouflaging and insulating the parents.

These early ancestors of birds developed more advanced techniques for caring for their young. They started to feed their young in the nest, pumping liquid food or placing small food items in their mouths. They also began to produce fewer and more dependent offspring and smaller eggs, and began nesting in bushes and then small trees to better protect their offspring from predators. Gradually the forelimbs of these creatures became feathered and even more elongated, enabling them to better manipulate their eggs and to "parachute" from their tree nests to a soft landing. Later they would develop the ability to glide and eventually fly by flapping their wings.

Adaptations for flight

The evolution of flight has endowed birds with many physical features in addition to wings and feathers. One of the requirements of heavier-than-air flying machines, birds included, is a structure that combines strength and light weight. One way this is accomplished in birds is by the fusion and elimination of some bones and the "pneumatization" (hollowing) of the remaining ones. Some of the vertebrae and some bones of the pelvic girdle of birds are fused into a single structure, as are some finger and leg bones all of which are separate in most vertebrates. And many tail, finger, and leg bones are missing altogether. Not only are some bones of birds, unlike ours, hollow, but many of the hollows are connected to the respiratory system. To keep the cylindrical walls of a bird's major wing bones from buckling, the bones have internal strut-like reinforcements (Harrison, 1957 and Nero, 1951).

The pneumatization of bird bones led to the belief that birds had skeletons that weighed proportionately less than those of mammals. Careful studies by Biggs and his colleagues (1957) have shown this not to be the case. More demands are placed on a bird's skeleton than on that of a terrestrial mammal. The bird must be able to support itself either entirely by its forelimbs or entirely by its hindlimbs. It also requires a deep, solid breastbone (sternum) to which the wing muscles can be anchored. Thus, while some bones are much lighter than their mammalian counterparts, others, especially the leg bones, are heavier. Evolution has created in the avian skeleton a model of parsimony, lightening where possible, adding weight and strength where required. The results can be quite spectacular: the skeleton of a frigatebird with a seven-foot wingspan weighs less than the feathers covering it.

Not all birds have the same degree of skeletal pneumatization. To decrease their buoyancy and make diving easier, some diving birds, such as loons and auklets, have relatively solid bones. Those birds are generally less skillful fliers than ones with lighter skeletons. Birds have found other ways to lighten the load in addition to hollowing out their bones. For instance, they keep their reproductive organs (testes, ovaries and oviducts) tiny for most of the year, greatly enlarging them only during the breeding season (Verheyen, 1953).

The respiratory system of birds is also adapted to the demands of flight. A bird's respiratory system is proportionately larger and much more efficient than ours as might be expected, since flight is a more demanding activity than walking or running. An average bird devotes about one-fifth of its body volume to its respiratory system, an average mammal only about one-twentieth (Akester, 1960).

The lungs of birds are less flexible, and relatively small, but they are interconnected with a system of large, thin-walled air sacs in the front (anterior) and back (posterior) portions of the body (Salt and Zeuthen, 1960). These, in turn, are connected with the air spaces in the bones. Evolution has created an ingenious system that passes the air in a one-way, two-stage flow through the bird's lungs. A breath of inhaled air passes first into the posterior air sacs and then, on exhalation, into the lungs. When a second breath is inhaled into the posterior sacs, the air from the first breath moves from shrinking lungs into the anterior air sacs. When the second exhalation occurs, the air from the first breath moves from the anterior air sacs and out of the bird, while the second breath moves into the lungs. The air thus moves in one direction through the lungs. All birds have this one-way flow system; most have a second two-way flow system which may make up as much as 20 percent of the lung volume (Sturkie *et al.*, 1962).

In both systems, the air is funneled down fine tubules which interdigitate with capillaries carrying oxygen-poor venous blood. At the beginning of the tubules the oxygen-rich air is in close contact with that oxygen-hungry blood; farther down the tubules the oxygen content of air and blood are in equilibrium (Frankel *et al.*, 1962). Birds' lungs are anatomically very complex but they create a "crosscurrent circulation" of air and blood that provides a greater capacity for the exchange of oxygen and carbon dioxide across the thin intervening membranes than is found in mammalian lungs.

Contrary to what was once believed, the rhythm of a bird's respiratory two-cycle pump is not related to the beats of its wings. Flight movements and respiratory movements are independent. The heart does the pumping required to get oxygenated blood to the tissues and to carry deoxygenated blood (loaded with carbon dioxide) away from them (Hering *et al.*, 1960). Because of the efficiency of the bird's breathing apparatus, the ratio of breaths to heartbeats can be quite low. A mammal takes about one breath for every four and one-half heartbeats (independent of the size of the mammal), a bird about one every six to ten heartbeats (depending on the size of the bird).

A bird's heart is large, powerful, and of the same basic design as that of a mammal. It is a four-chambered structure of two pumps operating side by side. One two-chambered pump receives oxygen-rich blood from the lungs and pumps it out to the waiting tissues (Durfee and Sturkie, 1963). The other pump receives oxygen-poor blood from the tissues and pumps it into the lungs. This segregation of the two kinds of blood (which does not occur completely in reptiles, amphibians, and fishes) makes a bird's circulatory system, like its respiratory system, well equipped to handle the rigors of flight (Giardina *et al.*, 1990).

The flight muscles of most birds are red in color ("dark meat") because of the presence of many fibers containing red oxygen-carrying compounds, myoglobin and cytochrome. They are also richly supplied with blood and are designed for sustained flight. Lighter-colored muscles ("white meat"), with many fewer such fibers, are found in pheasants, grouse, quail, and other gallinaceous birds. These are also well supplied with blood, are apparently capable of carrying a heavy work load for a short time, but fatigue more rapidly. If a quail is flushed a few times in a row, it will become so exhausted it will be incapable of further flight (Swank *et al.*, 2002).

Although birds have found many ways to streamline, lighten, or totally eliminate unnecessary parts (like urinary bladders), they have not stinted on nervous systems. Birds have brains that are proportionately much larger than those of lizards and comparable, in fact, with those of rodents. The brain is connected to sharp eyes, and has ample processing centers for coordinating the information received from them. A bird's nerves can rapidly transmit commands of the brain to the muscles operating the wings. It is the combination of visual acuity, quick decision making, and high-speed nerve transmission along short nerves that permits a Golden-crowned Sparrow to weave rapidly among the branches of a thicket, escaping the clutches of a pursuing Sharp-shinned Hawk (Pumphrey, 1961).

The mechanics of bird flight

The mechanics of bird flight are extremely complicated-more so than for an aeroplane with fixed wings-and some aspects are still not fully understood. When gliding, a bird wing is behaving like an aeroplane wing and lift is generated by its forward movement through the air, but in flapping flight the wing is acting both as lifting surface and propeller. In fast, level flight the wing performs a fairly simple up and down motion, starting at an angle of 60^0 above the horizontal and ending just below the horizontal. Forward movement comes from the outer part of the wing beating up and down rather than rotating like and aeroplane propeller (Burton, 1985).

The key to bird flight is the flight feathers ability to change shape automatically through the wing beat cycle. The trailing edge of the vane of each primary feather is broader and more flexible than the leading edge so, when forced down, the vane twists, the trailing edge goes up, and air is forced backwards to produce a forward thrust. To fly faster the bird either flaps its wings faster or more powerfully, to make the primaries twist more and increase the thrust (Beis and Newsholme, 1975).

Many birds can hover for a short time while picking an insect off a leaf or choosing a landing place; but few can sustain it for long since it is an energy draining exercise. The American black-shouldered kite spends only two and a half hours hunting by hovering each day, but this accounts for half its energy budget prolonged hovering is mainly limited to specialists such as the kestrels of the Old and New Worlds, but even these birds do no more than fly steadily into a light wind as they lack the muscle power to hover properly in still air (Kvist *et al.*, 2001). The humming birds are the most specialized of hovering birds; the structure of their wings is different from all other birds, except the closely related swifts. The bones of upper arm and forearm reduced in size so that the wing is almost all 'hand'. The elbow and wrist joints are practically immobile, and the wing acts as a rigid paddle rotating at a very flexible shoulder joint. When hovering, the body is held at an angle of 45^0 and the wings sweep through a narrow figure of eight in a horizontal plane. On the 'down stroke' the wings are angled to provide lift, and at the end of the beat the wing turns over, so presenting the same angle of attack and obtaining the same lift (Schultz *et al.*, 2001). The thrust on up strokes and down strokes is equal and opposite, so the humming bird stays in the same place, while a slight change in the angle of attack on either stroke will send the humming bird forwards or backwards.

To touch down gently, a bird must lose height and slow down, yet maintain enough lift to keep control and prevent a crash. Fanning the tail and changing to deep wing beats, as in take-off, help to slow the bird and also provide extra lift. If landing on a perch further braking can be achieved by approaching from below and flying up so that gravity is helping to check the forward momentum. Ducks and geese 'whiffle' when they drop sharply to land: they tumble and slide from side to side so air spills between the widely spread primaries, with a clear tearing sound, shedding enough lift to lose height quickly and the turning the wings into air brakes before hitting the ground. Sometimes they twist on to their backs and so lose lift completely (Rayner, 1985).

The chief flight muscles

Pectoralis major and supracoracoideus (pectoralis minor)

Some fifty different striated muscles and muscle slips have an action on the feathers and bones of the wing. Of these, pectoralis major and supracoracoideus (pectoralis minor) play the major role in elevating and depressing the humerus and thus the wing as a whole. Both these muscles arise from the ventral surface of the sternum thus, the centre of gravity remains low.

The bulky belly of pectoralis major converges and inserts by fleshy fibers, typically surrounded by dense tendinous envelope. On the ventral surface of the deltoid crest of the humerus the pectoralis muscle, therefore, depresses the humerus.

The flesh fibers of supracoracoideus (pectoralis minor) pass upward and converge on one or more large tendons, which pass dorsal through the triosseal canal. The tendon turns outward to insert on the dorsal surface of the humerus, usually just distal to its articular head. The bony margin of the triosseal canal functions as a pulley, so that the contraction of the ventrally situated belly of supracoracoideus elevates the humerus and the wing.

Pectoralis major is much larger than supracoracoideus in most birds that have been studied. Pectoralis major is said to be 15 times as large as supracoracoideus in the Black-backed Gull and, according to Nair (1954), 22 times in the pariah Kite. In some humming birds, however, pectoralis major is only 1.7 times as large as the supracoracoideus (Stolpe and Zimmer, 1939). Greenwalt (1960) observed that pectoralis major has ten times the weight of the small pectoral. The scatter from the mean is considerable due of course to the variability in relative weight of the small pectoral muscle. Hartman (1961) compiled a mass of new data on the weights of bird muscles, and concluded that supracoracoideus is largest in those birds that make a quick take off. It is especially small in cuculids, tytonids, and strigids. He found that supracoracoideus ranged from about 0.40 per cent of the body in species of the genus Buteo to 11.5 per cent of the body in trochilids, or 1.8 to 30 per cent respectively of the flight muscles.

Greenwalt (1960) concluded that for the entire procession of birds, from a tiny kinglet to a mute swan, pectoralis major averages 15.5 per cent of the body weight with very little scatter on either side of the mean. Hartman (1961) found variation in the ratio of pectoral weight to body weight in the same family; 10 to 17% in herons, 12 to 22% in flycatchers and 14 to 21% in swallows.

In sustained flying birds, protein loss during long-distance flight affect predominantly the breast muscle pectoralis and supracoracoideus, the digestive organs including liver and the skin (Akesson *et al.*, 1992; Battley *et al.*, 2000; Biebach, 1998). Before long flight bouts, several species have been shown to enlarge their pectoral muscles (Davidson and Evans, 1988; Dietz *et al.*, 1999; Driedzic *et al.*, 1993; Fry *et al.*, 1972; Lundgren *et al.*, 1995; Marsh 1984; Mc Landress and Raveling, 1981).

Flight capacity in non-moulting birds is determined mainly by total body mass and breast muscle size (penniquick, 1975). Body mass decreases continuously during flight, so birds could minimize their total energy expenditure by reducing their breast muscle tissue. Experiments with red knots flying in a wind tunnel indeed showed a close relationship between body mass and pectoral muscle thickness (Lindstrom *et al.*, 2000).

Schwilch *et al.* (2002) investigated the aspects of protein loss during migratory flight and formed that the reduction in breast muscle mass is a metabolically induced constraint or an adaptation to the decrease in body mass during flight. It has been found that during long distance flight protein source present in the flight muscle and leg muscles can be catabolised. Bauchinger and Biebach (2001) have analyzed samples of flight and leg muscle tissue from migratory garden warblers at three different stages of migration, i.e., Pre flight, post flight and recovery for its mass changes. They found that during migration the loss of mass occurs in flight and leg muscles. The changes in body mass were closely related to the changes in flight and leg muscle mass, suggesting that the skeletal muscles are involved in the protein metabolism associated with migratory flight. Biochemical analysis show that following flight a selective reduction occurred in the myofibrillar component of the flight muscle.

The adaptive value of size changes in different organ and muscle groups was studied in red knots in relation to their migration by Piersma *et al.* (1999). Birds were sampled on five occasions two times during subsequent refueling, at departure toward, and on return from, the high arctic breeding grounds. Their body mass increased without no sex differences in body and fat mass gains. Over the first interval, lean masses of heart, stomach and liver increased. During the middle time sizes of leg muscles, intestine, liver and kidneys increased. Stomach mass decreased over the same interval. The muscles and liver became smaller too, but pectoral muscles and heart increased in size. Sizes of "exercise organs" such as pectoral muscle and heart were best correlated with body mass whereas sizes of organs used during foraging (leg muscles) and nutrient extraction (intestine, liver) were best correlated with rate of mass gain. Kidneys changed little before take off, which suggests that they are needed as such during flight as during refuelling.

Lindstrom *et al.*(2000) studied the short term changes in the pectoral muscle size of captive red knots (*Calidris eanutus*) using ultrasonic imaging to monitor. Pectoral muscle thickness changed rapidly and consistently in parallel with body mass changes caused by flight, fasting and fuelling. Four knots flew repeatedly for 10hr periods in a wind tunnel over this period, pectoral muscle thickness decreased in parallel with the decrease in body mass. The change in pectoral muscle thickness during flight was indistinguishable from that during periods of natural and experimental fasting and fuelling. The body mass-related variation in pectoral muscle thickness between and within individuals was not related to the amount of flight, indicating that changes in avian muscle do not require power-training as in mammals. Their study suggests that it is possible for birds to consume and replace their flight muscles on a time scale short enough to allow these muscles to be used as part of the energy supply for sustained flight.

Jones (1982) investigated the pectoralis muscle from growing house sparrows using linearn, gravimetric, histological and ultrastructural techniques. Fibers in the pectoralis muscle of birds about to fledge were similar in composition to fibers in muscles from fully fledged juveniles. The inefficient flight of newly fledged house sparrows may be accounted for by the size of the pectoralis muscle relative to total body weight.

Power output from the flight muscles can be controlled by altering the strain rate (wing beat frequency or amplitude) or the proportion of muscle fibers used (Rayner, 1985; Rayner and Ward, 1999). Both these alternatives affect muscle efficiency. If the flight muscles are optimized to give maximum efficiency at one mechanical power output, efficiency will be reduced as power output changes away from this optimum. Adjustment of flight muscle size and the use of intermittent flight have been suggested to be adaptations for maintaining maximum muscle efficiency as body mass varies owing to build up and use of fuel stores during migration (Pennycuick, 1975; Rayner, 1985). Red knots do not use intermittent flight, but they do adjust flight muscle size to their current weight measured by ultrasound in a parallel study of the same birds (Lindstrom *et al.*, 2000). Great knots (*Calidris tenuirostris*), near relatives of the red knots, reduce flight muscle size during long migratory flights (Battley *et al.*, 2000).

The pectoralis: histology and histochemistry

The red and white muscle types

There are red and white muscles is common knowledge. Every one is familiar with the white breast meat and the pale-red leg meat of the chicken. The chicken pectoralis muscle is often referred to as an example of white muscle and the pigeon pectoralis as of the red variety. The redness of the muscle is due to the hemoglobin present in the cell themselves, and not due to the pervading blood, has long been known.

It should be emphasized that classifying muscles as red or white from a superficial examination is doomed to be an erroneous procedure. What is really important and essential is first of all to know the exact nature of its cell components. Such an approach is judicious for limited histological observations and it becomes all the more necessary as a basis for conducting detailed biochemical investigations requiring considerable precision and accuracy. The pigeon pectoralis muscle and the mammalian diaphragm, for instance, have been and still are, used as experimental material in several biochemical investigations.

On a cursory examination, the chicken pectoralis muscle appears to be constituted of only the white type of fibers. Recently this muscle has been shown histochemically to consist of two types of fibers, one containing myoglobin and the other without (Chandra Bose *et al.,* 1964). The myoglobin containing fibers are very few in number, whereas those that lack myoglobin predominate. The pigeon pectoralis on the other hand, has predominantly the myoglobin containing fibers and considerably fewer myoglobin-lacking fibers (Chinoy, 1963). The diameter of the myoglobin-containing fibers is much less than that of the other type of fibers. In sharp contrast to both the fowl and pigeon pectoralis muscles, that of the sparrow has only the narrow myoglobin containing fibers. However it should be stated that there are variations in the amount of the myoglobin present in different fibers.

On the basis of the myoglobin content, it is possible to distinguish two types of fibers in the pectoralis muscle of birds (1) Myoglobin containing fibers and (2) Myoglobin-lacking fibers. It is possible to characterize the avian pectoralis also on the same basis. (1) White mixed, e.g. Domestic fowl (2) Red mixed e.g. Pigeon, and (3) Red unmixed e.g. Humming bird and sparrow. There is however, no record of a white unmixed pectoralis in any bird. It should be of considerable interest to know whether such a pectoralis exists at all in any vertebrate higher than fishes.

The pectoralis muscle is a bipinnate muscle. The outer connective tissue covering is the epimysium, from its inner surface some septa penetrate in to the muscle, dividing the muscle irregularly into compartments called fasciculi. These septa forming the outer boundary of the fasciculi constitute the perimysium. Each fasciculus holds together a boundly of muscle fibers. The loose network of connective tissue extensions from the perimysium that envelop each muscle fibre is called the endomysium.

The muscle fiber has a thin outer covering called the sarcolemma. The contractile structure of the fiber consists of muscle columns, myofibrils, and myofilaments. A myofibril is seen to consist of transverse bands which differ in optical density and birefringence. The alternating array of thick, dense and anisotropy filaments are called A bands, while the thin less dense, less birefringent and isotropic filaments in the intervening areas are called the I bands. These I bands are bisected by Z membranes. In the middle of the A band only thick filaments are present. This region is called the H Zone. On either side of the H zone, the thick and thin filaments inter digitate and the optical density in this region is greatest.

Immediately below the sarcolemma are found the nuclei. They may be found deeper in the fiber among the myofibrils. In the sarcoplasm, there are five main components; sarcoplasmic matrix, mitochondria or sarcosomes, sarcoplasmic reticulum, sarcoplasmic lipid bodies, and the golgi apparatus.

Matheiu-Costello *et al.* (1998) examined fiber capillarization and ultrastructure in the highly aerobic flight muscle of six gray crowned rosy finches living at altitude (A) compared to eight sea-level (SL) house finches of the same subfamily, Carduelinae. Capillary to-fiber surface ratio and fiber mitochondrial volume were both greater in A, but their ratio was similar to SL, indicating a proportional increase in the size of the capillary to fiber interface and fiber mitochondrial volume in A to sustain high levels of aerobic capacity while living at altitude. Mathieu-costello *et al.* (1996) again noticed the consistent differences exist in the size of the capillary network for the size of the mitochondrial compartment in highly aerobic red muscle of tuna compared with bird and mammal.

Gille *et al.*(1998) showed a post hatching transformation of fast twitch oxidative-glycolytic (FTO) to fast-twitch glycolytic (FTG) fibers in the breast muscle in domestic chickens and turkeys. FTG fibers were first detected at 20 days of age. Thereafter, the fiber composition hardly changed. When distinguishable by enzyme histochemistry FTG fibers were already larger in diameter than FTO. There were only gradual differences in the growth pattern. FTG fibers, however, showed much higher absolute, percentage, and relative growth rates. The radial growth of myofibers is slow when compared to other one dimensional measurements. Fiber size differences are mainly due to a higher growth rate in FTG fibers, whereas the growth patterns show only minor differences.

The flight muscle types of the mallard duck (*Anas platyrhynchos*), the common coot *(Fulica atra)* and the yellow-legged gull (*Larus cachinnans*) were analysed morphometrically by Torrella *et al.*, (1998). Oxidative fibers always had significantly smaller size than anaerobic fibers, although no differences in number of capillaries per fiber were found. Fast oxidative fibers of the pectoralis and the triceps of the gull had greater sizes than the fast oxidative fibers of the mallard and the coot, which correlates with the difference in energetic demands between flapping and gliding flight. Greater fiber cross-sectional areas and perimeters seem suited to afford the long-lasting activity with low metabolic demands reviewed during gliding. By contrast, mallards and coots attain a high oxidative metabolism, during sustained flapping flight, by reducing fiber size at the expense of a diminished ability for force generation. The need to manage sustained swimming abilities effectivelly may result in similar fast oxidative glycolytic fiber morphometry of the hindlimb muscles studied, indicating that a compromise between the oxygen flux to the muscle cell and the development of power is highly optimised in oxidative fibers of the bird species studied.

It has been found that the space travel directly induces skeletal muscle atrophy Vandenburg *et al.* (1999). Space travel causes rapid and pronounced skeletal muscle wasting in humans that reduces their long-term flight capabilities. Space travel may also causes muscle atrophy indirectly by altering circulating levels of factors such as growth hormone, glucocorticoids, and anabolic steroids and/or by a direct effect on the muscle fibers themselves. Experiments conducted on aboard the space shuttle showed that significant muscle fiber atrophy occurred due to a decrease in protein synthesis rates without alterations in protein degradation. Return of the muscle cells to Earth stimulated protein synthesis rates of both muscle specific and extracellular matrix proteins relative to ground controls. These results show for the first time that skeletal muscle fibers are directly responsive to space travel and should be a target for countermeasure development.

Wada *et al.* (1999) studied the fiber type composition of 12 skeletal muscles in pigeon and chicken by staining for myofibrillar adenosine triphosphatase (ATPase) and succinate dehydrogenase (SDH). The muscles contained three types of muscle fibers. FG (fast-twitch glycolytic), FOG (fast-twitch oxidative glycolytic), and SO (slow-twitch oxidative). The numbers and diameters of the different types of fibers were examined. The muscles of chickens and pigeons consisted mainly of FG and FG + FOG fibers, respectively. In pectorals superficialis (PS) and latissimus dorsi (LD), which produce flapping movements in pigeons, some clusters of FG fibers were observed among FOG fibers and the diameter of FG fibers was more than twice as large as that of FOG fibers.

Pigeon pectoralis

The first clear exposition of the histological structure of the pigeon pectoralis was given by Bullard (1912). He described the mitochondria and the fat globules as distinct entities and as normal components of the muscle cell. He also recognized the fact that these fat inclusions differed considerably in different muscles as well as in the different fibers of the same muscle. George and Jyothi (1955) studied the histology of the pectoral muscle of the pigeon and some other birds and a bat. They confirmed Bullards observations on the pigeon breast muscle in its having two types of fibers, a broad white fiber and a narrow red fiber. They concluded however that the fat present as globules in the red fiber is the main source of energy for flight and that Bullard's suggestion of its being reserve food material is only a secondary purpose.

The early electron microscope studies of the pigeon pectoralis were done by Harman (1955). Weinreb and Harman (1955) also opposed the generally accepted concept of mitochondrial structure since they could not obtain evidence for the presence of enveloping membranes in the pigeon pectoral muscle mitochondrion.

On the basis of cytochemical distribution of oxidative enzymes (succinic dehydrogenase and cytochrome oxidase), Howatson (1956) and George and Talesara (1961 a) defined the two types of fibers in the pigeon pectoral muscle using the light microscope. The location of the lipid bodies and mitochondria was shown by Howatson (1956) in an electron microscope study of the pigeon pectoralis muscle. The mitochondria are present in between myofibrils as in the fowl pectoral muscle (Bennett and Porter, 1953), but in the pigeon pectoralis they are considerably more numerous and larger in size. That the basic nature of the pigeon breast muscle mitochondria is not unlike that of mitochondria from other sources was thus established. The lipoid nature of the lipid bodies was also confirmed by him.

The nuclei of the pigeon pectoralis muscle have not been studied in detail. Observations under the light microscope show that the nuclei of the white fibers occur in the matrix inside the contractile part of the fiber and are larger than those of the red fiber, in which the nuclei are found in the matrix just beneath the sarcolemma. It is also noticed that there are prominent areas of clear nucleoplasm in the nuclei of the white fiber. The sarcoplasmic reticulum is more prominently seen in the white fibers when subjected to suitable staining techniques under the light microscope.

The effects of short and long term exercise on intracellular glycogen and fat in pigeon pectoralis was studied by Parker and George (1975) in an attempt to determine the functional role of the component broad white and narrow red fiber during activity. Short term stimulation resulted in a selective depletion of intracellular glycogen stores from white fiber populations whereas prolonged long term stimulation resulted in the resynthesis of glycogen within these fibers and a concomitant reduction in the intracellular fat and glycogen reserves in most red fibers. It was postulated that during flight the white fibers indulge only in brief bursts of intense physical activity such as in quick take-off, rapid accelerations or sudden maneuvers and utilize glycogen as fuel whereas the red fibers perform sustained activity as increasing flight and metabolize chiefly fat.

Parker and George (1976) stimulated the pigeon pectoralis *in vivo* at a high intensity of 25V and found that continuous stimulation resulted in the depletion of intracellular glycogen from both fiber types and a simultaneous reduction in the intracellular fat stores of the red fibers. While glycogen provides the only source of energy for the white fibers, glycogen as well as fat serves as fuel for the red fibers. They have suggested that though fat is the major fuel for the red fibers, their glycogen reserves are utilized to ensure a steady supply of oxaloacetate essential in the oxidation of fatty acids via the Krebs cycle, and that fatty acid catabolism helps to maintain gluconeogenesis.

It has been found that the citrate inhibits the activities of phosphofructokinase from muscles and nervous tissues from different animals across the animal Kingdom except for the insects (Newsholme *et al.*, 1977). Inhibition was observed with the enzymes from both aerobic pectroal muscle of pigeon and anaerobic fish muscle, and pectoral muscle of the game birds.

Hasse *et al.* (1986) examined the influence of long distance flight on corticosterone secretion in trained homing pigeons. The concentration of corticosterone was increased in comparison with the levels in nonexercised controls. The increase in corticosterone concentration was unrelated to the distance flown or duration of flight.

The studies of Srivastava *et al.* (1978) demonstrated significant molecular differences in the myosin derived from the flight muscles of a flying pigeon and a non flying fowl. Myosin was purified from the flight muscles of a pigeon and a fowl and found that the specific activity is higher for the fowl. $CaCl_2$ concentration of myosin of both pigeon and fowl is similar. However, the two proteins differ in their interactions with ADP, ATP and P-chloromercribenzoate.

Giladi *et al.*(1997) examined urinary water loss and plasma levels of arginine vasotocin (AVT) in free-flying, tippler pigeons trained to fly continuously. Plasma levels of AVT increased after flight to between three and eight times the preflight levels. Their data are consistent with previous studies of dehydration in birds and exercise in mammals in which both increased filtered water reabsorption (FrH2O) and decreased glomerular filtration rate contribute to renal conservation of water.

The red and white fiber types of pigeon pectoralis

The two types of fibers are easily distinguished even in untreated fresh frozen sections of the pigeon pectoralis. The diameter of the broad fiber is 69.00 ± 14.00 µ and that of narrow 30.11 ± 6.56 µ (George and Naik, 1959a). The former shows clear sarcoplasm whereas the latter is studded with granular inclusions of mitochondria, and lipid globules (George and Naik, 1959b).

The Pectoralis: biochemical aspects

a) Myoglobin

It was shown histochemically that the pale fibers of the fowl pectoralis and the red fibers of that of the pigeon contain myoglobin (Needham, 1926). The concentration of myoglobin in a muscle is generally regarded as an index of the capacity of the muscle for aerobic metabolism and sustained activity. Lawrie (1950) found that leg muscle (gastrocnemius) of fowl there is about 10 times as much myoglobin as in its pectoralis, while the pigeon pectoralis contains about 4 times as much as in the muscle of the leg. It was also observed that the myoglobin content of pigeon pectoralis (0.25%) is about 40 times as great as that of the fowl pectoralis (0.006%).

In the transport of oxygen by hemoglobin or myoglobin and in the transport of electrons to oxygen by the cytochrome system, the porphyrin ring, with its iron moiety, is the key structure. Nair (1952) estimated the iron content in the pectoralis and supracoracoideus muscles of some Indian birds and observed that the latter muscle contained considerably less iron than the former except in the domestic fowl. Highest figures were obtained for the muscles of the sparrow and the least for the domestic fowl and the domestic duck. The kite and the pigeon muscles were close seconds. The high content of iron in the pectoralis muscles of the sparrow and the kite is caused by the fact that these muscles contain only red fibers. The reason why the figures obtained for the pigeon pectoralis were less than those of the sparrow is because this muscle, as already mentioned, is a mixed one containing the white myoglobin-lacking fibers. In this context the recent observations of Talesara (1961) on the pigeon pectoralis are of interest. The iron content was found to vary at different depths of the muscle in accordance with the relative distribution of the red and white fibers.

It is known that at the venous pressure of oxygen, oxymyoglobin is less dissociated than oxyhemoglobin. This greater affinity of myoglobin for oxygen is of special significance in the physiology of the avian muscle because it facilitates oxygen transfer from oxyhemoglobin to the sites of oxidation in the muscle cell. Myoglobins prepared from different species of vertebrates, including a cormorant and pelican, were studied by Brown *et al.* (1962). They found that the fish myoglobins contain free sulfhydryl groups but not the avian and mammalian myoglobins.

Locomotory muscles of the tufted duck, *Aythya fuligula* were analysed by Turner and Butler (1988) for mass aerobic and anaerobic enzyme activities, fiber type proportions, capillarity, mitochondrial and myoglobin content. The estimated aerobic capacity of the muscles correlated well with the muscle's maximal oxygen uptake both when measured during swimming and when predicted for steady-state flight. The delivery of oxygen to mitochondria is facilitated by the dense capillarity and high myoglobin content of the muscles.

Thomas (1985) measured the myoglobin concentration and myosin ATP use activity in the pectoral muscle of wild spruce grouse *(Dendragapus canadensis)* and ruffed grouse (*Bonasa umbellus*), together with the weight of the Pectoralis major, superacoracoideus and heart. Myosin ATP activities were similar in both species, but spruce grouse contained 15 times more myoglobin in the pectoralis muscle and the heart was three times heavier than that of the ruffed grouse. The relative mass of the flight muscles and wing loading were similar between species characteristics of the pectoral muscle of both grouse species reflect adaptations to predation and advertising displays. The glycolytic nature of the ruffed grouse pectoral muscle and small heart size is an adaptation to a sedentary existence within a small home range. The more oxidative pectoral muscle of spruce grouse together with its larger heart are adaptations to seasonal dispersals requiring more sustained flight.

b) Fuel

In the performance of muscular work and the maintenance of muscle tone energy is expended. This energy is generated from a chain of chemical reactions supported by a continuous supply of fuel. Carbohydrates and fats constitute the fuel reserves in all muscles, but properties of these two fuels differ in different muscles depending on the nature of their activity. The chief fuel store in the white fibers of the pigeon pectoralis is glycogen whereas in the red ones it is fat. George and Naik (1959) estimated separately the glycogen content of methanol preserved red and white fibers of the pigeon pectoralis isolated by teasing them out manually with watchmaker's forceps under a binocular dissection miscroscope. It is seen that the glycogen content is highest in the most superficial and deepest regions of the muscle where there are more white fibers, whereas the fat content is highest in the middle of the dorsoventral axis of the muscle where the number of the red fibers is the highest. Although white muscles contain more glycogen, red muscles contain more fat. For sustained activity fat is a better fuel because it yields double the energy and metabolic water obtained from glycogen.

In migratory birds the glycogen and fat content of the pectoralis muscle are almost doubled at the time of migration (Vallyathan, 1963; Vallyathan and George, 1964a). The main fuel used by migrating birds is fat, which is laid down at stopover sites enroute (Alerstam and Lindstrom, 1990). As carrying large fat stores incurs increase in flight cost and predation risk (Alerstam and Hedenstrom, *et al.*, 1998; Kullberg *et al.*, 1996) most bird species accumulate only small fat deposits and refuel at several successive stopover sites. But to cross large ecological barriers such as the Gulf of Mexico or the Sahara Desert, fuel loading needs to be much greater and some birds have been found to double their mass before crossing the Sahara which involves flight distances of at least 1,500 km (Fry *et al.*, 1970).

Many mechanisms reduce the cost of muscle activity and thereby fuel use. Conley and Lindstedt (2002) describe a set of specializations that reduce the cost of contraction in the high frequency twitches that are used by a wide variety of animals for either sound production or flight. Minimizing the cost of these contractions means that cellular ATP production can meet ATP demand and sustain the high contractile rate. Two classes of specialization are found that minimize the contractile cost. The first class reduces the muscle work required per contraction. Light appendages such as rattles, insect limbs and membranous wings that require little work for movement are used in high-frequency contractions. The second set of specializations involves processes that minimize energy use. High frequency muscles tend to have a lower cross bridge content, fewer attached cross-bridges and shorter length changes per contraction. The result is low muscle-specific forces (stress), small length changes (strain) and rapid contraction times that suggest that these muscles push the lower limit of contractile function. The consequence of function at this lower extreme of contraction is to minimize the contractile cost of high frequency muscles. Thus specializations that permit rapid contractions at a low rate of ATP use per twitch are the basis of a minimization strategy for energy saving in muscles contracting at high frequency.

c) Metabolism

It is seldom realized that the foundations of earlier knowledge of aerobic metabolism were laid by studies in which the pigeon breast muscle was used as the experimental material. The observations of scientists showed minced pigeon breast muscle respires very actively by the complete oxidation of pyruvic acid, producing little or no lactic acid stimulated a rapid flow of important investigations, culminating in the monumental work of Hans Krebs. Krebs showed that, when pyruvate and oxaloacetate were incubated together with the minced muscle under anaerobic conditions, an accumulation of considerable amounts of citric acid resulted. These discoveries enabled him to postulate in 1937 the cycle of reactions called the tricarboxylic acid cycle (Krebs cycle), which brings about the oxidation of pyruvic acid to carbon dioxide and water (Krebs, 1954).

The rates at which different animals use energy have been calculated on the basis of their oxygen consumption at the stress condition (Chai and Dudley, 1996). The consumption is then used to calculate the basal metabolic rate, which is expressed as the number of kilocalories of energy used per kilogram of body weight, per hour. Small birds have proportionately larger surfaces (through which heat is lost) in relation to their mass of metabolizing tissue than do large birds. A Bushtit can maintain a body temperature like a Tundra Swan's because it has such a higher basal metabolism (i.e., uses proportionately more energy). Hummingbirds, with their tiny bodies and high levels of activity, have the highest metabolic rates of any animals roughly a dozen times that of a pigeon and a hundred times that of an elephant. To maintain those rates, hummers have to consume about their weight in nectar daily. In fact, a warm-blooded animal can't be smaller than a hummer or a shrew. Further reduction in size would make it impossible for the creature to eat fast enough to maintain its body temperature (Weimskirch *et al.*, 2001).

The basel metabolic rates of nonpasserine birds are very similar to those of some mammals (Dawson *et al.*, 1983). Passerines, however, tend to have 30-70 percent higher metabolic rates than either nonpasserines or mammals, for reasons that are not understood.

It is now well established that mitochondria contain the complete enzymatic machinery for the operation of the Krebs cycle and for the generation of high energy phosphate bonds. The respiratory activity of different types of muscle has been correlated with the mitochondrial density and the color of the muscle (Paul and Sperling, 1952). Respiratory activity, expressed as μmole O_2 per hour per gram wet weight of the tissue (when α-ketoglutarate was used as substrate), was found to be highest in the pigeon breast muscle (with highest color and mitochondrial density) among the breast muscles of the three birds studied (pigeon; Mallard; and chicken).

In the light of the histochemical and biochemical observations on the red and white muscles, as well as red and white fibers within muscles, it is clear that the red muscle with its red fibers is adapted for aerobic metabolism and that the white muscle and white fibers are adapted for anaerobic metabolism. The predominant metabolite in the red muscle fibers is fat whereas in the white muscle fibers it is glycogen. Obviously, fat should be the chief fuel for energy in the red fibers and glycogen in the white (Gilmour, 1960).

It was shown by Weinhouse *et al.* (1950) that the pigeon breast muscle is capable of metabolizing fat. George and Jyoti (1957) studied the relative reductions in fat and glycogen in an exercised pigeon pectoralis muscle and estimated that over 70% of the energy expended is derived from fat. George and Vallyathan (1964) have shown that when the pectoralis muscle of a pigeon is electrically stimulated, fat in the form of free fatty acids is transported to the muscle from the liver and adipose tissue. Histochemical study of the pectoralis muscle of the rubythroated hummingbird revealed that the prominent sarcoplasmic reticulum inside the muscle fibers stain for lipids Chandra-Bose and George, 1964. They have, therefore, indicated that lipids are transported through the sarcoplasmic reticulum to the mitochondria for oxidation.

If fat is to be utilized for energy, it has first of all to be broken down to fatty acids and glycerol so that the fatty acids released can undergo β-oxidation. George and Talesara (1962) demonstrated that the pigeon breast muscle is incapable of direct oxidation of triglycerides. They, therefore, suggested that fats will have to be acted upon first by the muscle lipase, which will split fat into the component fatty acids as the first step for the utilization of fat. Muscle lipase capable of hydrolyzing glycerides of long-chain fatty acids has been localized in the mitochondria of skeletal muscles (Bokdawala and George, 1964). George (1964) has suggested that a higher lipase capable of hydrolyzing esters of long-chain fatty acids resides in the mitochondria and that another lipase, which acts on those of short-chain fatty acids, is present in the microsomal fraction (103.84 μmole CO_2 per hour per mg protein), the next being in the mitochondrial fraction (47.05 μmole CO_2 per hour per mg protein). From these observations it was suggested that the function of the microsomal lipase is the esterification of fatty acids with glycerol to form neutral lipids, and that perhaps it also effects the splitting up of fat into fatty acids thereby rendering the products of hydrolysis suitable for oxidation by the mitochondria (Bennett and Porter, 1953).

It should be of considerable interest to know how the two types of bird muscle fibers with two different metabolic adaptations, but existing side by side in the same muscle, function. The red fibers by virtue of their heavy fat load and dense cellular inclusions indulge in sustained tonic contractions. The white ones, on the other hand, possess the necessary qualities for rapid tetanic contractions. George and Nene (1965) have shown that when the pigeon breast muscle is electrically stimulated the white fibers are completely depleted of their glycogen store within a few minutes of continuous contraction, but this is not true of the red fibers. These observations indicate that the white fibers, by their rapid contractions, should enable the bird to have a quick take-off and also to manipulate sudden turns while in flight. The red fibers on the other hand, indulge in slower contractions and in sustained flight. It is probable that during continuous flight the white fibers remain passive.

A muscle which indulges in sustained activity requires a continuous generation of high energy compounds. Such a muscle has a high myoglobin content and cytochrome oxidase activity but a low ATP (adenosine triphosphate) and creatine phosphate content and low myofibrillar ATPase activity (Lawrie, 1953a, b). On the other hand, a muscle capable of fast contractions for short periods of time utilizes energy mainly from its store of ATP and creatine phosphate. According to Lawrie (1953a, b), a muscle of this kind has low myoglobin content and cytochrome oxidase activity but high ATP and creatine phosphate content and high myofibrillar ATPase activity. The same characteristics are applicable to the red and white fibers, respectively, of the pigeon pectoralis muscle. The different forms of the enzyme ATPase, which hydrolyzes the terminal phosphate of ATP to release chemical energy, is known to occur in muscle in three different forms: mitochondrial, myofibrillar, and sarcotubular (George, 1962).

Totzke *et al.* (1998) studied the substrate utilization and regulatory mechanisms of metabolism in migratory garden warblers by measuring plasma levels of glucose, free fatty acids (FFAs), beta-hydroxybutyrate, insulin and glucagon in response to oral glucose loads. Three different physiological states were examined: (a) the autumnal migratory period on a high and (b) on a fasted low body mass level, and (c) the postmigratory period with low body mass. It showed that during premigratory and migratory periods glucose utilization may be inhibited by a more favorable oxidation of fatty acids as would be predicted by the glucose fatty acid cycle. However, the inhibition of glucose utilization seems to be counterregulated by stronger responses of insulin and glucagon. These results may be important also in the consideration of food selection during premigratory periods and refueling abilities of birds crossing ecological barriers.

Frasier (1984) explained a theory explaining the relationships between metabolic rate and body mass for birds and mammals is developed in terms of the mitochondrion theory of aerobic metabolism, the sliding filament theory of muscle contraction, simple models of vertebrate anatomy and activity, and other propositions. Both body mass and metabolic rates are shown to result from a homogeneous vertebrate design quantitatively expressed in terms of a set of nearly invariant parameters with five degrees of freedom: propulsion technique, mitochondrion capability, non-skeletal muscle mass exponent, characteristic skeletal length, and sturdiness factor. The first three of the degrees of freedom are phylogenetic group specific. The last two vary considerably even within a single species. The theory is shown to agree satisfactorily with placental mammal, marsupial mammal, passerine bird and nonpasserine bird data. Algorithms for determining metabolic rates and body mass as functions of skeletal characteristic length are developed and quantitative estimates of the constants occurring in the algorithms are given.

Suarez *et al.*(1986) studied the metabolic sources of energy in hummingbird flight. The measurement of maximum activities of key enzymes of carbohydrate, fat and amino acid catabolism in flight muscle and heart of rufous hummingbirds (*Selasphorus rufus*) revealed the high ATP requirements of short-term hovering flight can only be supported by the oxidation of carbohydrates. Fat oxidation can support only the low energetic requirements of long-term foreword or migratory flight. Mitochondria isolated from flight muscle oxidize pyruvate and palmitoyl-CoA. The inhibition of pyruvate oxidation by palmitoyl-CoA oxidation indicates that fat oxidation inhibit carbohydrate oxidation in transition from short-to long-term flight. Surarez and his colleagues (1990) studied the fuel selection in rufous hummingbirds. Their flight muscles possess sufficient maximal activities of hexokinase and carnitine palmitoyltransferase for the use of either glucose or long-chain fatty acids as metabolic fuels. Respiratory quotients indicate that fatty acid oxidation serve as the primary energy source in fasted resting birds. In 1992 Suarez studied the resting and maximal mass-specific metabolic rates scale inversely with the body mass. Maximal capacities for oxygen and substrate delivery to muscle mitochondria, as well as mitochondrial oxidative capacities of small hummingbirds shows at the upper limits to mass-specific metabolic rate. Suarez and Gass (2002) showed that hummingbirds display some of the highest known mass-specific rates of aerobic metabolism among vertebrates. High enzymatic flux capacities through pathways of carbohydrate and long-chain fatty acid oxidation indicate that substrate can fuel flight. Hummingbird rely on fat to fuel migratory flight, short foraging bouts are fueled by oxidation of carbohydrates.

BIOCHEMICAL STUDIES ON BIRD FLIGHT

Thesis submitted to the University of Calicut
for the Degree of Doctor of Philosophy
under the Faculty of Science

By

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Materials and Methods

MATERIALS AND METHODS

The experimental animal

Pigeon, *Columba livia*, is the living symbol of peace all over the world. Pigeon belongs to the Phylum Chordata, Class Aves and Order Columbiformes. It is one of the most commonly used laboratory experimental bird.

Field characters: Familiar slaty grey bird with glistening metallic green, purple and magenta sheen on neck and upper breast. Two dark bars on wings and a band across the end of tail. Sexes alike in appearance. It is free living and aerial in mode of life.

Habits: The wild pigeon lives in holes of rocks, caves and roofs of buildings. The domestic pigeon lives mostly in places like grain markets, godowns, mosques and temple towers.

Food: Cereals, pulses, groundnuts, etc.

Call: A deep gootr-goo, gootr-goo.

Nesting: Season-undefined, practically all year in semiferal birds. Nest a flimsy collection of a few sticks on a ledge or in a fissure of cliff, or on rafters and ceilings of dwelling houses, deserted or occupied.

Egg: Two, white, elliptical. Both sexes share all the domestic duties.

Life cycle of pigeon

The life cycle of pigeon begins with eggs laid by female pigeon on any available ledge. Female pigeons lay normally a pair of eggs. After a three weeks of incubation period, new born hatches out and immediately fed on special "pigeon milk," which is a curd like substance produced from the crops of both the parents. There is a drastic change in the external features of newborn pigeon (squabs), the grey feathers gradually replace the yellow down feathers. The squabs become independent at about 2 months old.

For experiments, male birds were used. The adult male birds of same age and size were collected from pigeon hatchery. The birds were reared in the laboratory under the similar conditions and fed with cereals, pulses and groundnuts. The rearing cage was cleaned twicely a day. The cage was disinfected with bleaching water in order to avoid spread of disease once in a week. Birds required for experiments were drawn from the cage just before the experiment.

Electrical stimulator

Electrical stimulator is an instrument used to give electrical stimulus of required impulse to pectoralis major muscle of pigeon, *Columba livia*. The electrical stimulator device was made in-house by the Instrumentation Department of University of Calicut.

It produces a current of 20 volt. The continuous flow of electrical current is controlled using mechanical regulator. The current was allowed to pass into the pectoralis major of the experimental birds using copper electrodes. A ringer solution containing 6g NaCl, 0.075g KCl, 0.1g $CaCl_2$ and 0.1g $NaHCO_3$ in 1000 ml distilled water was used for immersing the dissected birds.

For experiments, male birds were caught and they were anesthetized with diethyl ether; the skin was removed and major flight muscles was carefully dissected out along with the spinal nerves attached to it. The whole muscle preparation was kept immersed in ringer solution. The right pectoralis major muscle was taken as 'normal'. The dissection was carried out and the fresh weight of dissected muscle from the right muscle was noted. The left pectoralis major muscle of the experimental bird was continuously excited for 30 minutes, in situ. This was done by piercing the nerves of the muscles with electrodes from a specially built electrical stimulator with 20 volt output. The electrically stimulated muscle preparation was taken as 'experimental'. During the electrical stimulation of the muscle it was immersed in ringer solution at 25°C. Pieces of muscle tissue were excised from the left pectoralis muscle immediately after the experiment and weighed.

The whole blood from the right and left brachial artery was collected using a syringe before and after the experiment.

Preparation of homogenates

A homogenate of a tissue is, by definition a suspension of the formed elements of the cells in a diluted cytosol milieu, which is isotonic with the cell contents and which is inert. Here water was used as the dispersion medium. Nevertheless, the word 'homogenate' will be retained for purpose of convenience. A hand operated whole glass device was used for homogenising the tissues.

Biochemical analyses

The crude homogenate of the muscle tissue was used for analysis. The whole blood collected from normal and experimental conditions was centrifuged at 1000 rpm for 5 minutes and the serum was used for the analysis. Tissue samples from 3 birds were pooled and used for the assays.

Estimation of total proteins

The proteins were precipitated with 10% sodium tungstate and 2/3N sulphuric acid and centrifuged for 10 minutes at 2000 rpm in a centrifuge. The precipitate formed was dissolved in 0.1N sodium hydroxide solution and aliquots were estimated for total protein according to Lowry *et al.* (1951) using crystaline bovin serum albumin (fraction V, Sigma) as standard.

Estimation of total free amino acids

The homogenates and blood plasma was precipitated with 80% alcohol and centrifuged at 2000 rpm for 10 minutes. Estimations of total free aminoacids in the samples were done by the method of Lee and Takahashi (1966). The colour developed was read at 540nm against the reagent blank in a spectrophotometer.

Estimation of individual free amino acids

The individual amino acids were determined using HPLC according to Ishida et al. (1981). The sample in the vials were carefully washed with 6 N HCl and transferred into the test tubes. The volume was made up to 5 ml and the tubes were heat-sealed after filling pure nitrogen gas. Hydrolysis was carried out in a hot air oven at 110°C for 24 hours. After hydrolysis, the contents were removed and filtered into round bottom flasks through Whatman filter paper. The flasks were flash-evaporated to remove traces of HCl and the process was repeated for three times with distilled water. The residue was made up to 1 ml with 0.05M HCl. The samples thus prepared were filtered again through a membrane filter of 0.45 µm and 20 µl was injected in to Shimadzu HPLC-LC 10. As having a column packed with a strongly acidic cation exchange resin, i.e., styrene divinyl benzene copolymer with sulfinic group. The column is of sodium type [ISC-07/s 1504 Na-Shimadzu]. The mobile phase consists of two buffers, Buffer A (Sodium citrate, absolute alcohol, perchloric acid, pH 3.2) and buffer B (Sodium citrate, boric acid, 4N NaOH, pH 10). The oven temperature was maintained at 60°C, the amino acids were eluted from the column by step wise elution, i.e., acidic amino acids first, followed by neutral and then finally basic amino acids. The eluted amino acids were detected by using a fluorescence detector after post column derivitization with O-phthalaldehide. In the case of proline and hydroxyproline, imino groups was converted to amino group with sodium hypochlorite.

Estimation of glucose

Glucose was estimated according to Nelson (1944) and Somogyi method (1951). The homogenates and blood plasma were deproteinized with 10% sodium tungstate and 2/3N sulphuric acid and centrifuged at 2000 rpm for 20 minutes. The resultant supernatant was used for the estimation and the colour developed was read at 540nm against a reagent blank in a spectrophotometer.

Estimation of urea

The urea in the muscle tissue and blood plasma was estimated using Fearon reaction, modified by Beale and Croft (1961). The homogenates and blood plasma were deproteinized with 80% alcohol and centrifuged at 2000 rpm for 10 minutes. The supernatant was treated with diacetyl monoxime-phenylanthranilic acid and then with activated phosphoric acid reagent and heated for 11 minutes. The colour developed was read after cooling, against a reagent blank in a spectrophotometer at 535nm.

Estimation of creatinine

Creatinine was estimated based on Jaffe reaction by Mc Fate, et al. (1954). The tissue homogenate and blood plasma were deproteinized with 10% sodium tungstate and 2/3N sulphuric acid and centrifuged at 2000 rpm for 10 minutes. The resultant supernatant was used for estimations and the colour developed was read at 520nm against a reagent blank in a spectrophotometer

Estimations of aspartate amino transferase (AST) and alanine amino transferase (ALT) activities

The activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were estimated following the method of Reitman and Frankel (1957) using sodium pyruvate as standard. The homogenate of muscle tissue and blood plasma were used for the enzyme assay. The substrate mixture of alpha oxoglutaric acid and L-aspartic acid for AST and the substrate mixture of alpha oxoglutaric acid and L-alanine for ALT were incubated with the enzyme source for one hour. The colour developed due to the formation of keto acid was read after 10 minutes at 520nm against a reagent blank in a spectrophotometer. One unit of enzyme activity corresponds to the formation of one μmole of ketoacid per minutes at 37^0C under the experimental conditions. The specific activity of AST and ALT enzymes was expressed as unit activity per mg crude tissue protein.

Estimation of protease activity

The assay system consisted of glycine – NaOH buffer, 50mM, pH 9.8, 0.20 ml of 5% (W/V) casein solution (weighed amount of casein, Merck, suspended in water and heated on a steam bath, with the addition of 0.1N NaOH drop by drop, until the dissolution was completed), 0.10ml enzyme preparation, in a total volume of 1.0ml. The incubation was carried out at 37^0C for 1 hour. The reaction was stopped by adding an equal volume of 0.3M trichloroacetic acid. The samples were centrifuges for 20 minutes at 4000 rpm. The total free amino acid liberated was estimated in the supernatant according to the method of Hori (1970) using Folin-Ciocalteau regent and tyrosine as standard. Controls were run simultaneously.

Unit of enzyme activity

One unit of enzyme activity corresponded to the formation of one micromole of the tyrosine under the conditions of the assay. Activity units were represented by the amount of tyrosine produced by the enzymatic action per milligram of muscle tissue /blood plasma.

Estimation of total lipids

Total lipids was estimated according to Bligh and Dyer (1959). One-gram tissue/1ml blood plasma was homogenated with 10 volumes of chloroform: methanol (2:1 v/v) mixture, without the addition of sand. The homogenate was refrigerated for 30 minutes and centrifuged. The supernatant was decanted off and the residue was rehomogenized with 10ml of chloroform: methanol mixture and centrifuged again. The supernatants were combined and shaken up with 4ml of 0.05N NaCl in a separating funnel. The separation of layers was allowed to proceed to completion overnight inside a refrigerator. The lower phase was drained off and made up to 10ml with chloroform. An aliquot was transferred to a container of known weight and the solvent evaporated at room temperature under a draught of air. The sample was dried to constant weight by storage over anhydrous calcium chloride in a vaccum desiccator.

Estimation of total carbohydrates

Total carbohydrate was estimated using anthrone method, according to Hedge and Hofreiter (1962). Muscle tissue homogenate and blood plasma were hydrolysed by 2.5 N- HCl and neutralized with solid sodium carbonate and centrifuged at 2000 rpm for 10 minutes. The supernatant was treated with anthrone reagent and heated for 8 minutes. After cooling the colour developed was read against a reagent blank in a spectrophotometer at 630nm.

1

BIOCHEMICAL STUDIES ON BIRD FLIGHT

Thesis submitted to the University of Calicut
for the Degree of Doctor of Philosophy
under the Faculty of Science

By

T.V. SHAJITHA

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, INDIA

2005

Results

550

RESULTS

BIOCHEMICAL ANALYSIS OF THE MUSCLE TISSUE AND BLOOD PLASMA OF *COLUMBA LIVIA* DURING NORMAL AND EXPERIMENTAL CONDITIONS

Total proteins

The concentration of protein in the muscle tissue and blood plasma of both normal and experimental birds are recorded in table 1 and Figure 1.

Table 1. Total protein content in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Total protein	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	27.46±4.12	33.57±5.43
Experimental	20.25±2.25	25.33±1.28

The values are the means of five determinations with standard deviations.

The concentration of total protein was lower in experimental conditions of both muscle tissue and blood plasma compared to the normal conditions of birds. In the experimental birds there was a 26 % reduction in the muscle protein whereas up to 24% fall in the plasma protein was recorded in the blood plasma compared to the normal.

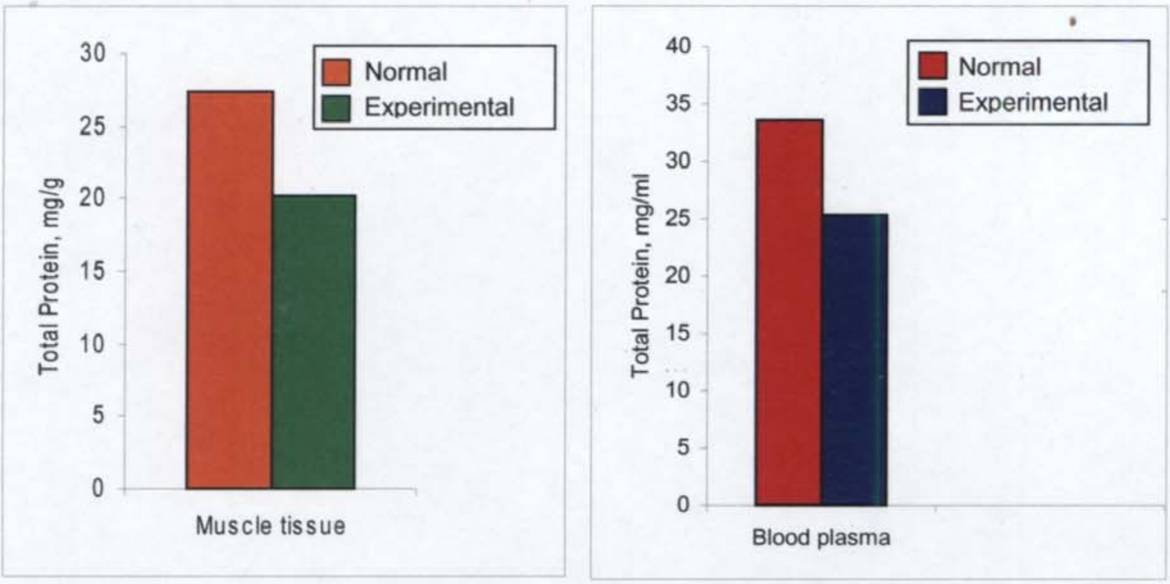


Figure 1. Total proteins in muscle tissue and blood plasma in normal and experimental birds

Total free amino acids

The concentration of total free amino acids in the muscle tissue and blood plasma of both normal and experimental birds are given in table 2 and figure 2.

Table 2. Total free amino acid content in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Total free amino acids	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	0.057±0.007	0.076±0.007
Experimental	0.071±0.006	0.086±0.010

The values are the means of five determinations with standard deviations.

The total free amino acid content was higher in experimental conditions of both muscle tissue and blood plasma of birds, compared to the normal conditions. The increase was striking in the case of both muscle tissue and blood plasma of experimental birds. There was a 24% increase in the titre of total free amino acid in muscle tissue in experimental birds whereas only 12% increase was noted in the case of blood plasma, compared to the normal conditions.

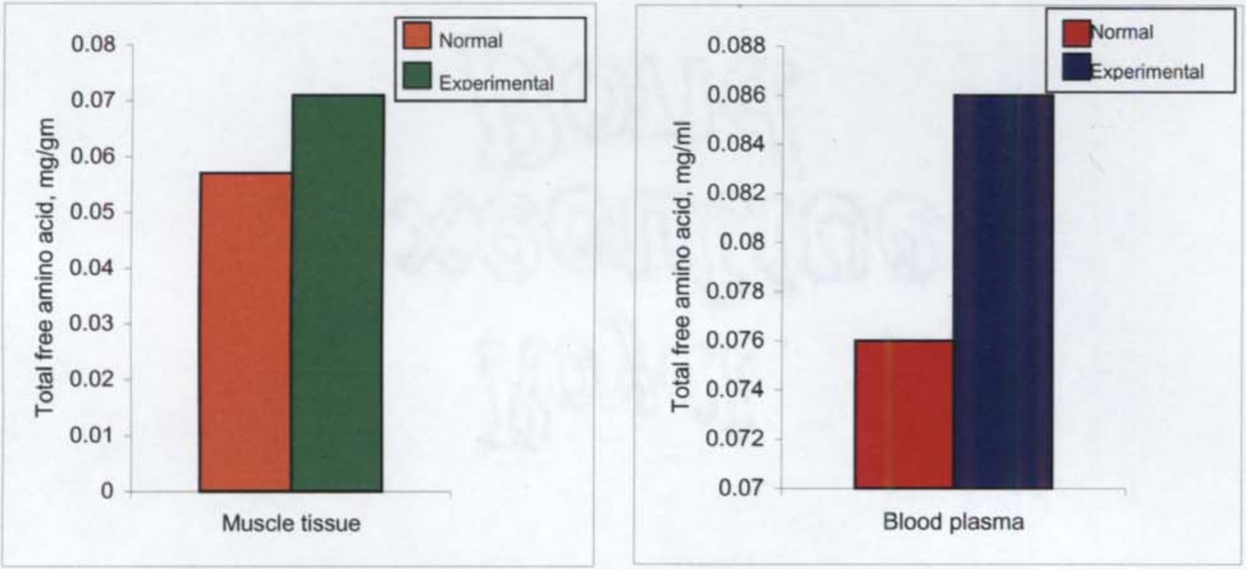


Figure 2. Total free amino acids in muscle tissue and blood plasma in normal and experimental birds

Individual free amino acids

The composition of each amino acid as percentage of total free amino acids of the muscle tissue and blood plasma of normal and experimental birds are given in the table 3, and figures 3a, 3b and 3c.

Table; 3 Figure 3a, 3b and 3c.

Table: 3. Composition of each amino acids as percent of total amino acids in the muscle tissue and blood plasma of *C. livia*

Amino acids	Muscle tissue		Blood plasma	
	Normal	Experimental	Normal	Experimental
Alanine	5.94	7.55	6.55	5.30
Arginine	0.39	1.80	2.30	7.57
Aspartate	0.92	1.42	3.95	3.61
Glutamate	1.38	0.97	8.07	8.04
Glycine	0.96	0.97	5.64	5.06
Histidine	35.68	27.59	13.32	9.87
Lysine	1.04	0.16	0.21	1.52
Phenylalanine	1.27	1.14	3.45	9.23
Proline	0.39	0.41	2.04	1.31
Serine	1.21	1.05	6.61	4.22
Taurine	47.73	53.45	28.18	39.32
Threonine	0.63	0.65	3.41	2.09
Tyrosine	2.17	2.20	13.04	0.66
Valine	0.28	0.56	3.14	2.13
Total	100	100	100	100

The values are the means of 2 determinations

A total of fourteen amino acids were separated from the muscle tissue and blood plasma and relative composition of each of them was determined. The following are the amino acids identified: taurine, aspartate, threonine, serine, glutamate, proline, glycine, alanine, valine, tyrosine, phenylalanine, histidine, lysine and arginine.

The percentage of each amino acids present in the total free amino acid was different in muscle tissue and blood plasma of normal and experimental conditions. In general, taurine is found to be the most abundant amino acid with 53.45% followed by histidine with 35.68%. The percentage of lysine is noted as the least.

The composition of free amino acids in the muscle tissue and blood plasma of normal birds was found to be much different from experimental conditions. The relative proportion of free amino acids in the tissues of normal and experimental birds are given below.

Muscle-normal

Taurine > histidine > alanine > tyrosine > glutamate > phenylalanine > serine > lysine > glycine > aspartate > threonine > proline > arginine > valine.

Muscle-experimental

Taurine > histidine > alanine > tyrosine > arginine > aspartate > phenylalanine > serine > glycine > glutamate > threonine > valine > proline > lysine.

Blood-normal

Taurine > histidine > tyrosine > glutamate > serine > alanine > glycine > aspartate > phenylalanine > threonine > valine > arginine > proline > lysine.

Blood-experimental

Taurine > histidine > phenylalanine > glutamate > arginine > alanine > glycine > serine > aspartate > valine > threonine > lysine > proline > tyrosine.

The percentage of aspartate, serine, proline, glycine, alanine, valine, tyrosin and the percent of arginine are higher and threonine, glutamate, phenylalanine, lysine and histidine are lower in the muscle tissue of experimental conditions with respect to the normal conditions. Percentage of phenylalanine, lysine and arginine are higher whereas aspartate, thereonine, serine, glutamate, proline, glycine, alanine, valine, tyrosine and histidine are lower in blood plasma experimental in contrast to blood plasma normal (Table 3).

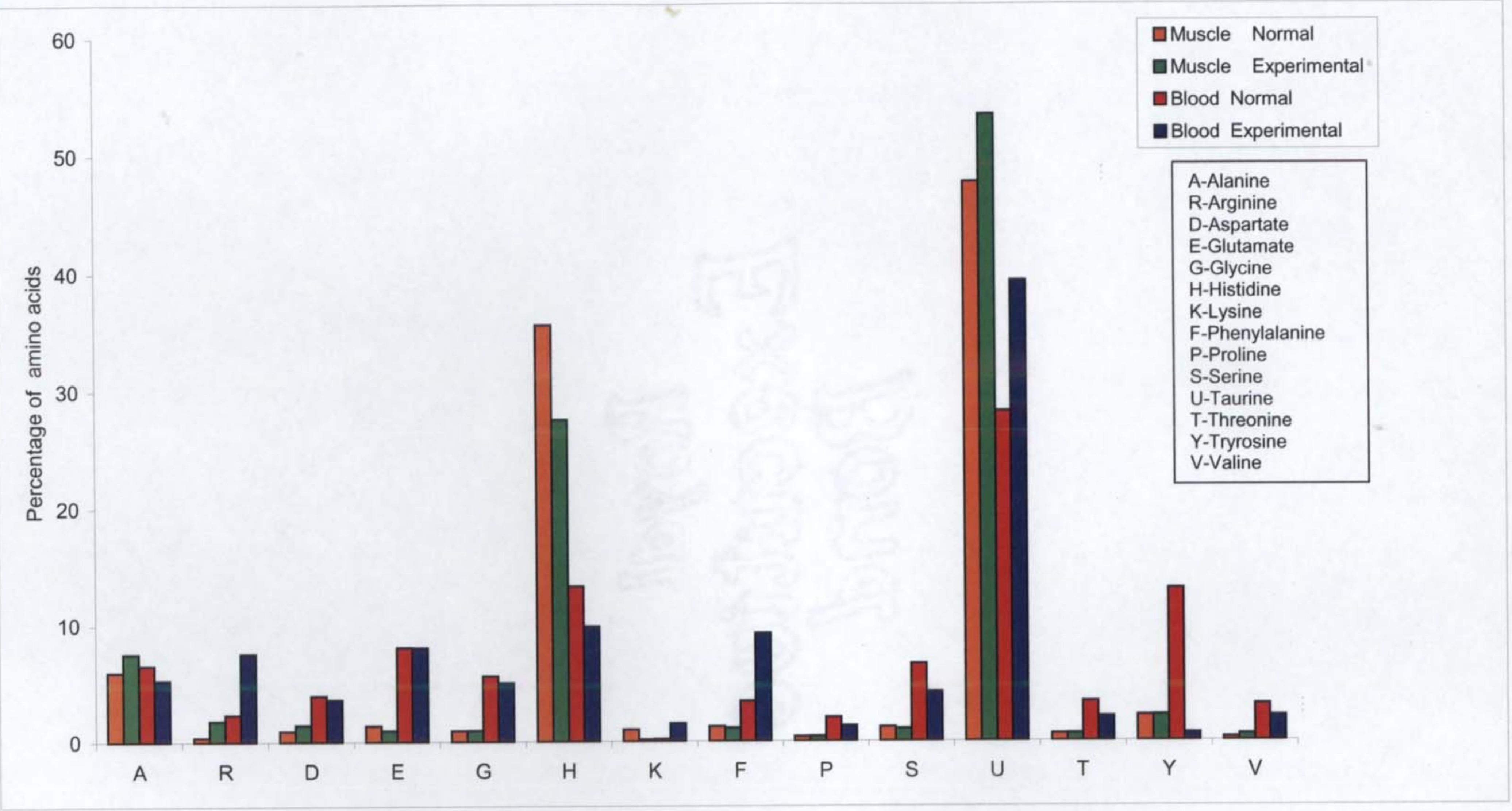


Figure 3a. Composition of each amino acid as percentage of total amino acids in the muscle tissue and blood plasma of *C. livia*

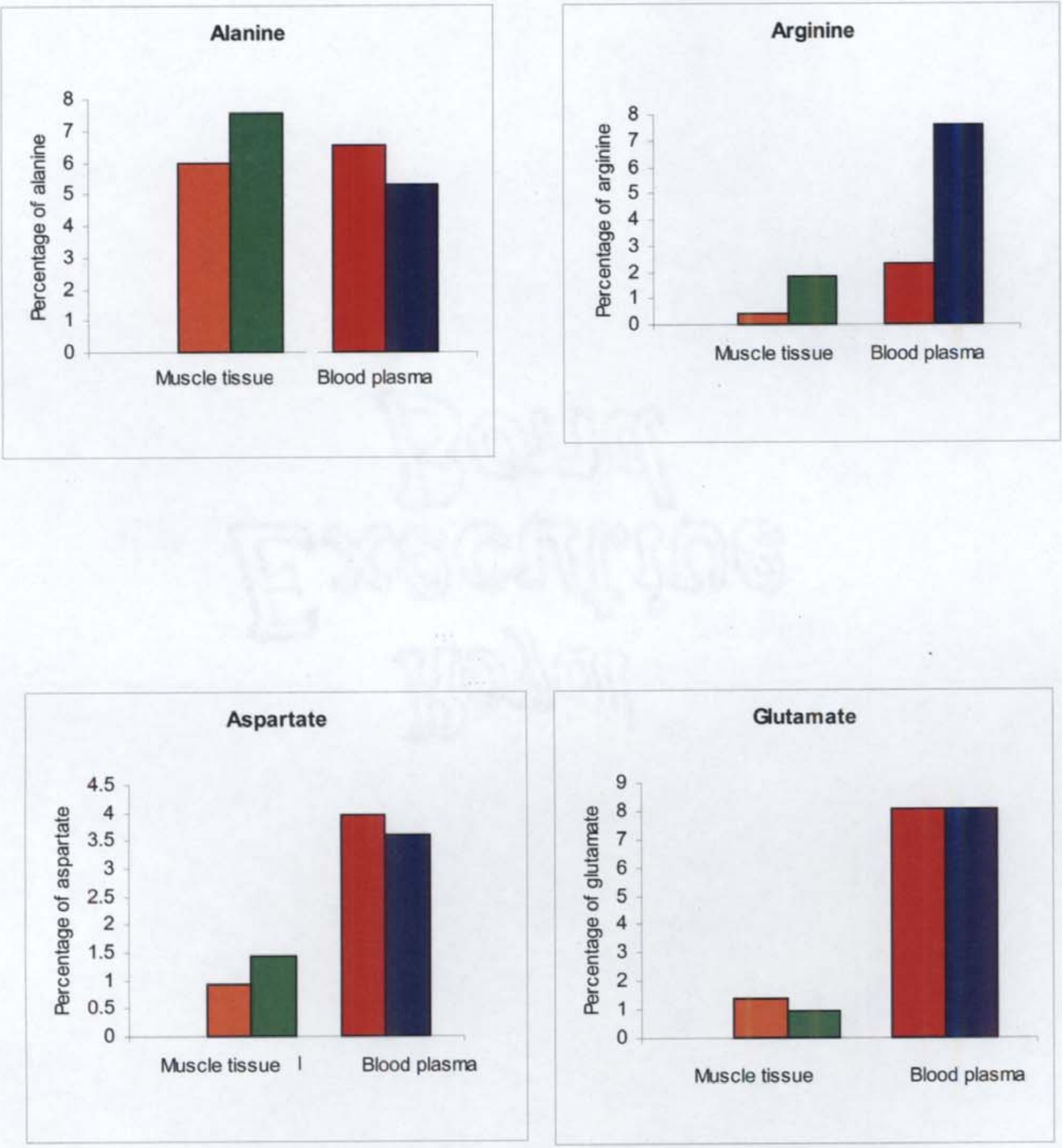


Figure 3b: 1-4. Percentage of individual amino acids of the total free amino acid in the muscle tissue and blood plasma of *C. livia*

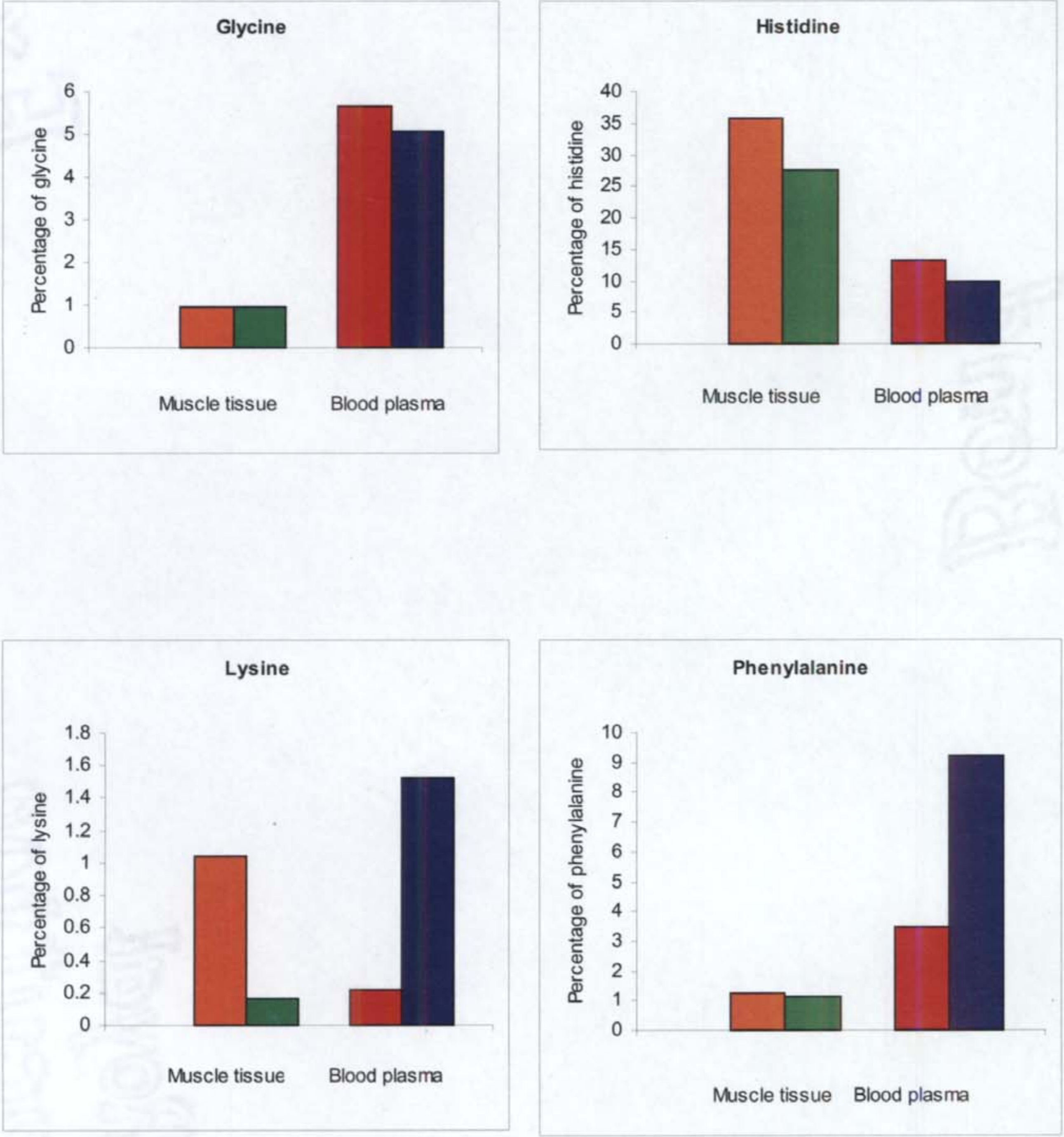


Figure 3b: 5-8. Percentage of individual amino acids of the total free amino acid in the muscle tissue and blood plasma of *C. livia*

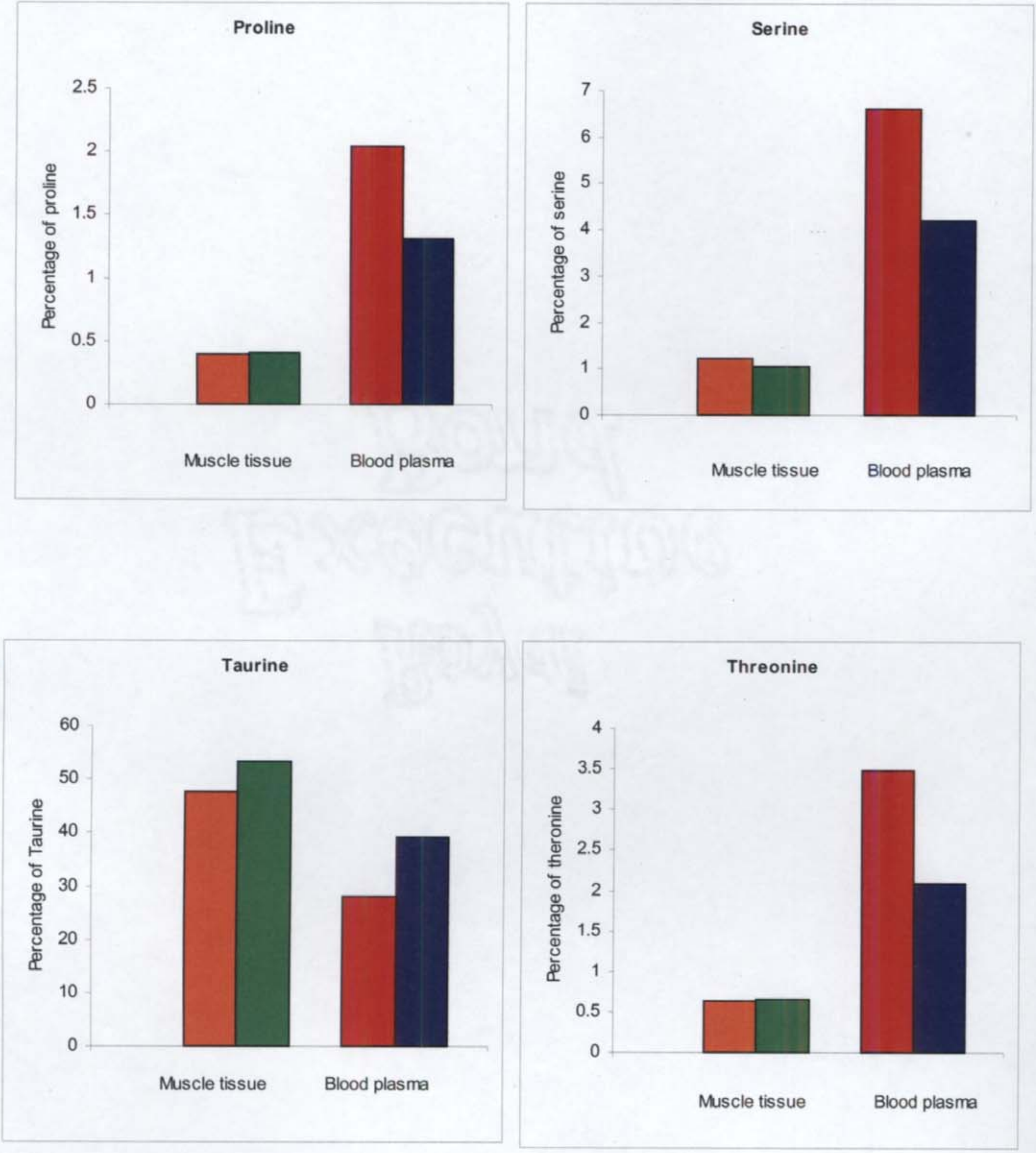


Figure 3b: 9-12. Percentage of individual amino acids of the total free amino acid in the muscle tissue and blood plasma of *C. livia*

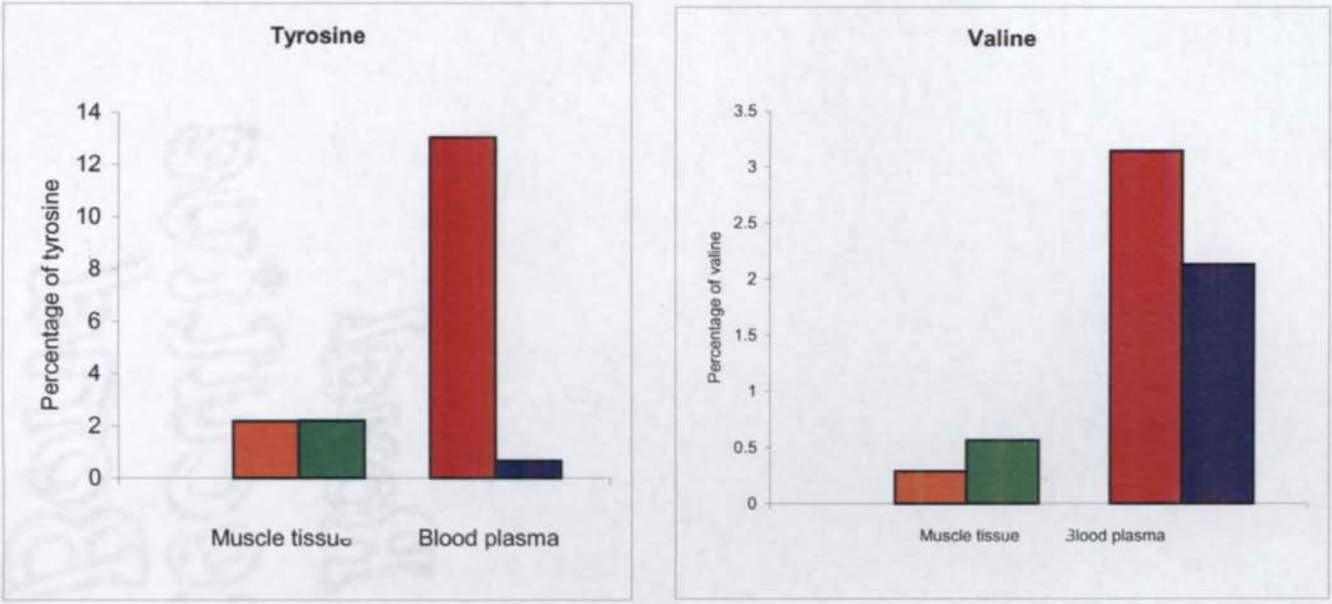
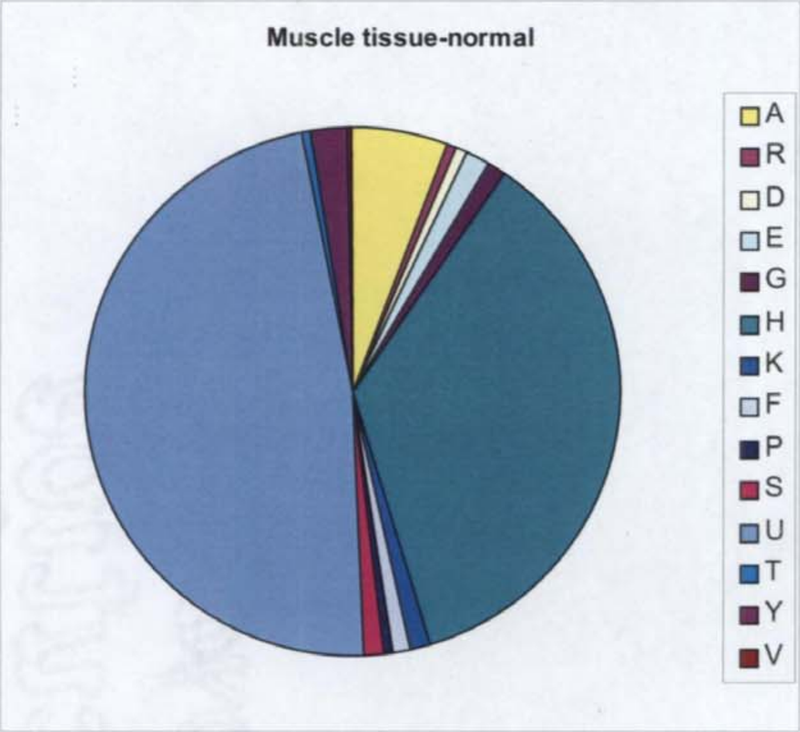


Figure 3b: 13-14. Percentage of individual amino acids of the total free amino acid in the muscle tissue and blood plasma of *C. livia*



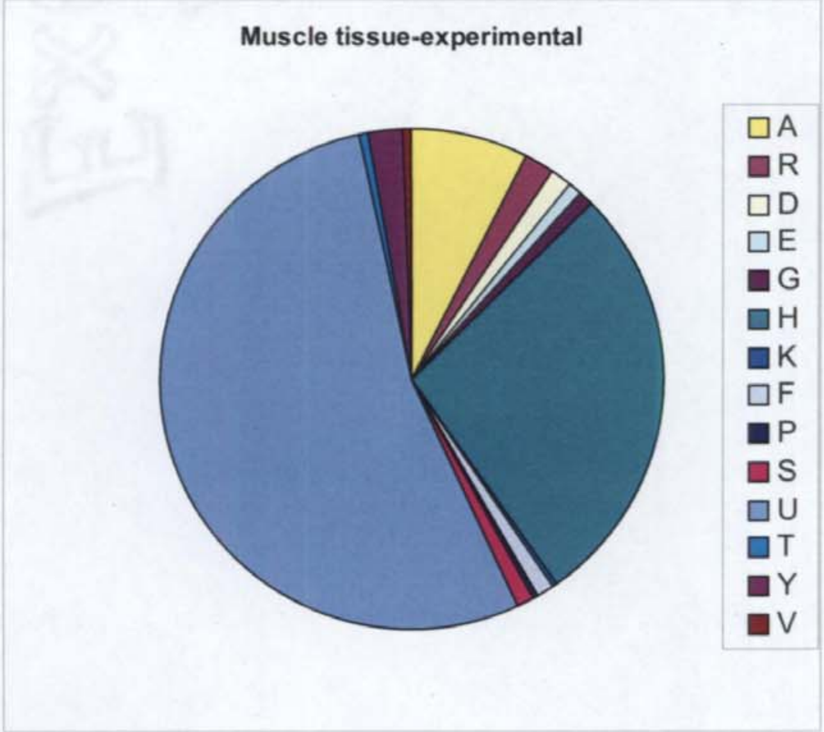
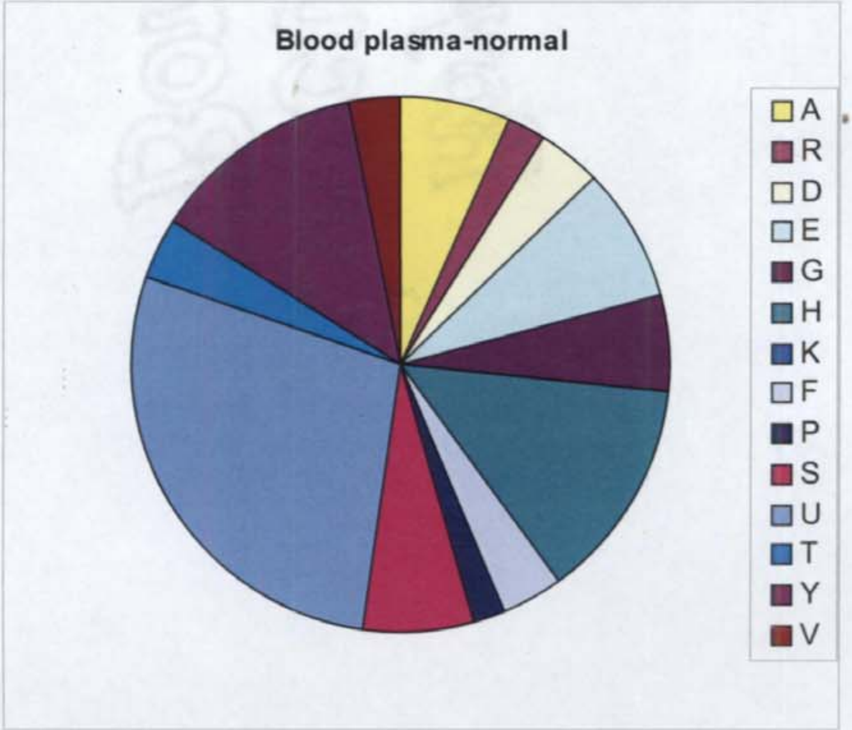


Figure 3c: 1-2. Composition of each amino acid as percent of total free amino acid in the muscle tissue of *C. livia* during normal and experimental conditions



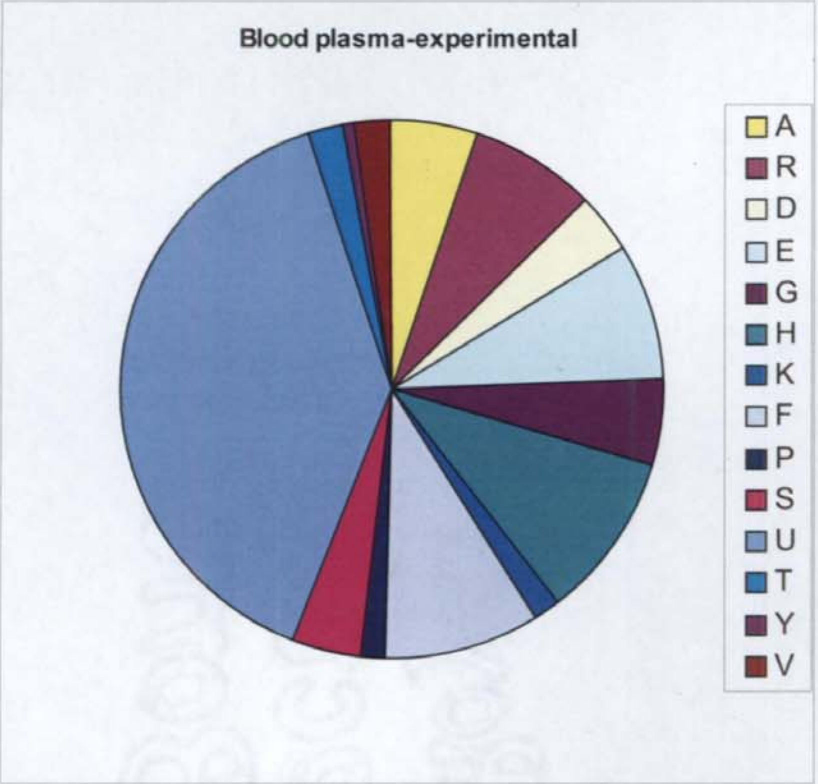


Figure 3c: 2-4. Composition of each amino acid as percent of total free amino acid in the blood plasma of *C. livia* during normal and experimental conditions

Total glucose

The concentration of glucose in the muscle tissue and blood plasma of both normal and experimental birds are recorded in table 4 and figure 4.

Table: 4. Glucose concentration in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Glucose	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	2.73±0.27	3.64±0.31
Experimental	4.15±0.17	5.04±0.63

The values are the means of five determinations with standard deviations.

Glucose concentration was higher in muscle tissue and blood plasma of experimental conditions compared to the normal conditions. There was a 52% and 38% increase in glucose concentration in experimental and normal conditions of birds respectively.

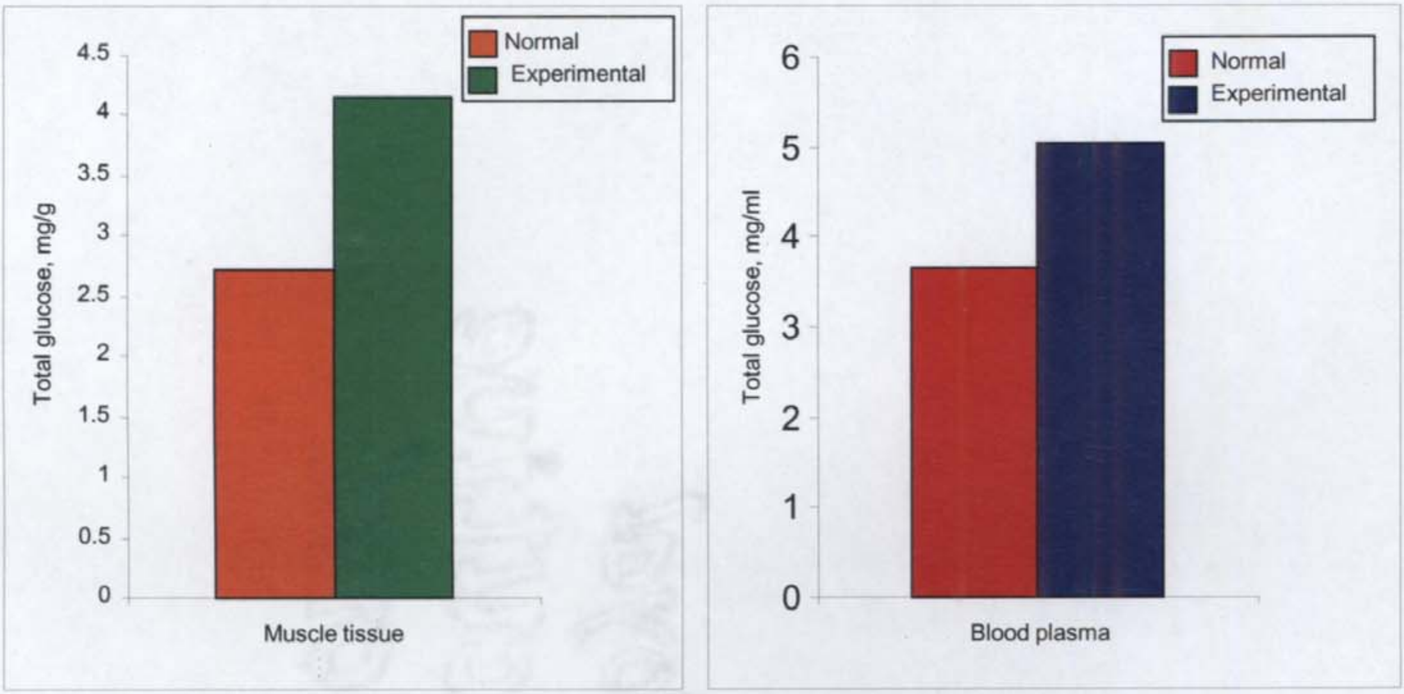


Figure 4. Glucose concentration in muscle tissue and blood plasma in normal and experimental birds

Urea

Urea levels in the muscle tissue and blood plasma of both normal and experimental birds are recorded in table 5 and figure 5.

Table: 5. Urea levels in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Urea	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	0.032 ±0.004	0.127 ±0.012
Experimental	0.050±0.001	0.151±0.015

The values are the means of five determinations with standard deviations.

Levels of urea showed a higher value in experimental conditions of both muscle tissue and blood plasma compared to the normal conditions of birds. In the experimental birds there was a 56% increase in the muscle urea whereas only 18% increase was noted in the case of blood plasma urea.

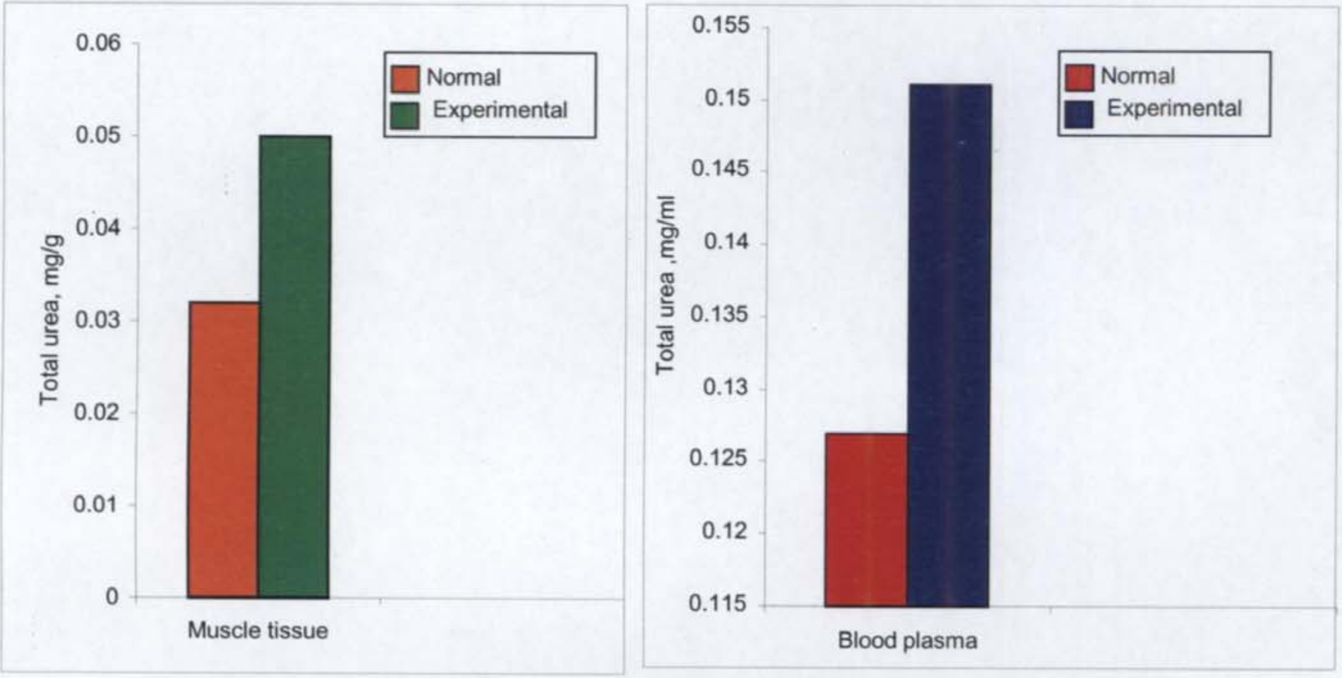


Figure 5. Urea levels in muscle tissue and blood plasma in normal and experimental birds

Creatinine

Levels of creatinine in the muscle tissue and blood plasma of both normal and experimental birds are recorded in table 6 and figure 6.

Table: 6. Creatinine levels in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Creatinine	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	0.014±.0.001	0.006±0.001
Experimental	0.019 ±0.003	0.009±.002

The values are the means of five determinations with standard deviations.

The levels of creatinine was recorded higher in experimental conditions of both muscle tissue and blood plasma compared to the muscle tissue and blood plasma of normal conditions. The increase of creatinine concentration in the experimental birds was 36% and 50% in the case of muscle tissue and blood plasma respectively.

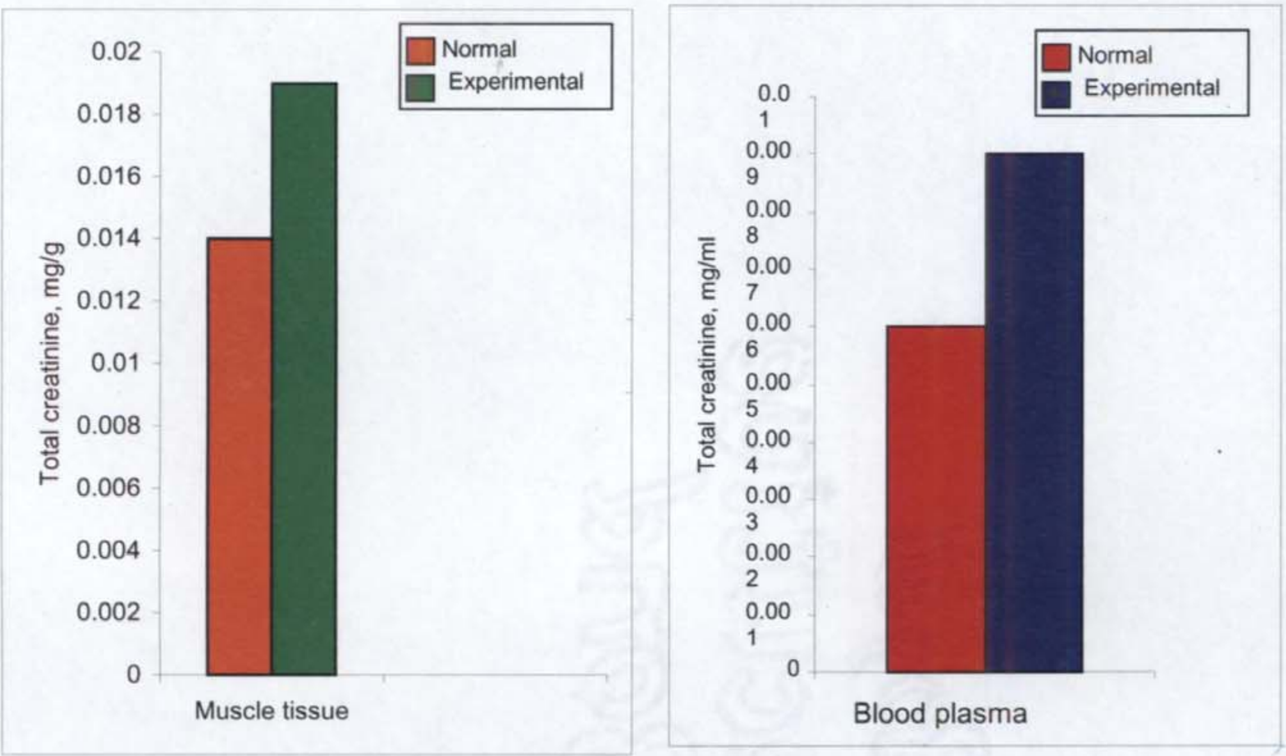


Figure 6. Creatinine levels in muscle tissue and blood plasma in normal and experimental birds

Alanine aminotransferase (ALT) activity

The enzymatic activity of alanine aminotransferase in the muscle tissue and blood plasma of both normal and experimental birds are presented in table 7 and figure 7.

Table: 7. Alanine aminotransferase activity in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Alanine amino transferase activity	
	Muscle tissue, units/g	Blood plasma, units/ml
Normal	6.28±0.84	15.06±2.46
Experimental	13.86±3.22	28.52±4.87

The values are the means of five determinations with standard deviations.

The ALT activity was increased in the experimental conditions of both muscle tissue and blood plasma compared to normal conditions. In the muscle tissue the ALT activity was more than double whereas in the blood plasma the increase was near to double.

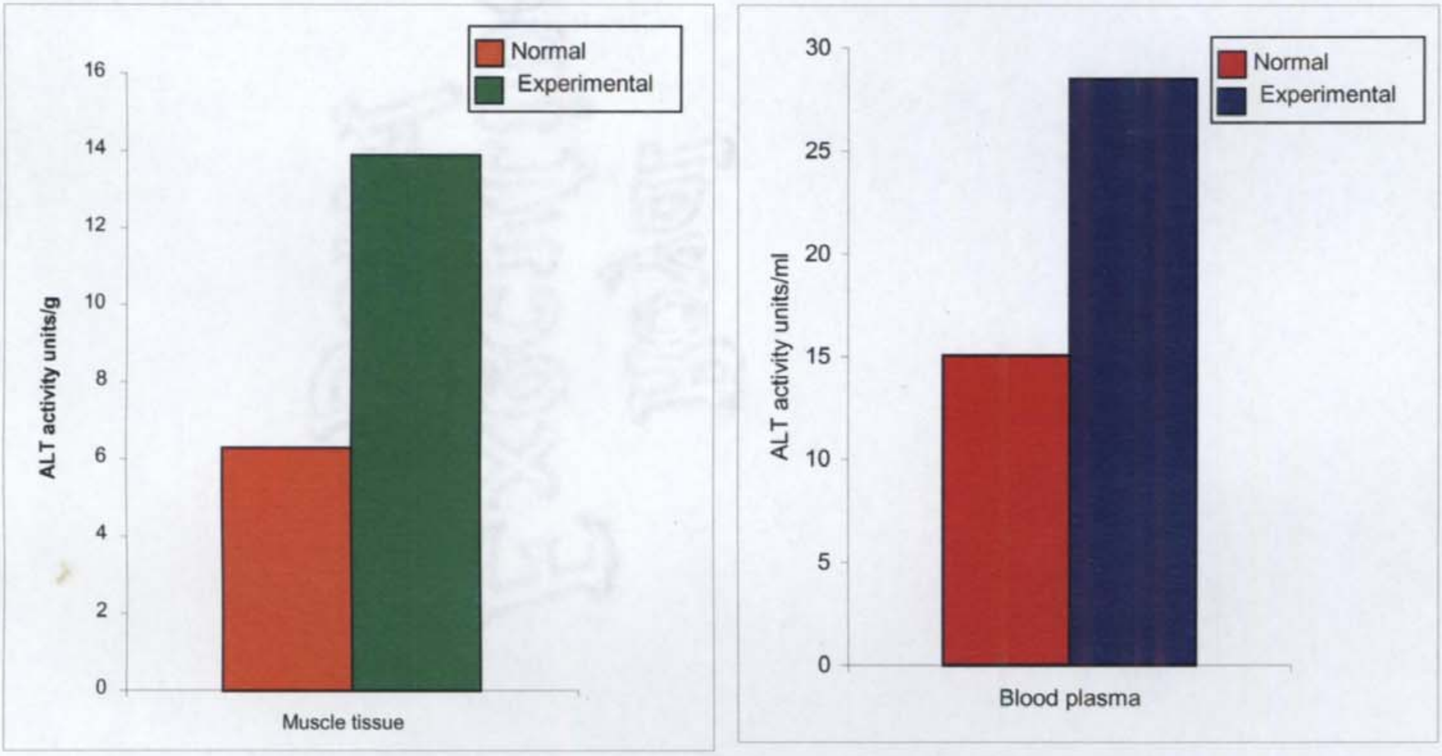


Figure 7. Alanine aminotransferase activity in muscle tissue and blood plasma in normal and experimental birds

Aspartate amino transferase (AST) activity

The enzymatic activity of aspartate amino transferase in the muscle tissue and blood plasma of both normal and experimental birds are presented in table 8 and figure 8.

Table: 8. Aspartate amino transferase activity in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Aspartate amino transferase activity	
	Muscle tissue, units/g	Blood plasma, units/ml
Normal	8.19±0.52	13.27±2.36
Experimental	5.28±0.98	8.14±0.47

The values are the means of five determinations with Standard deviations.

The changes in the enzymatic activity of AST in the experimental bird tissues were not similar to the activity of ALT. There was an inverse correlation in the activity of AST in the tissues of experimental birds with that of ALT activity. The reduction in AST in experimental birds was 35% and 38% in the case of muscle tissue and blood plasma respectively.

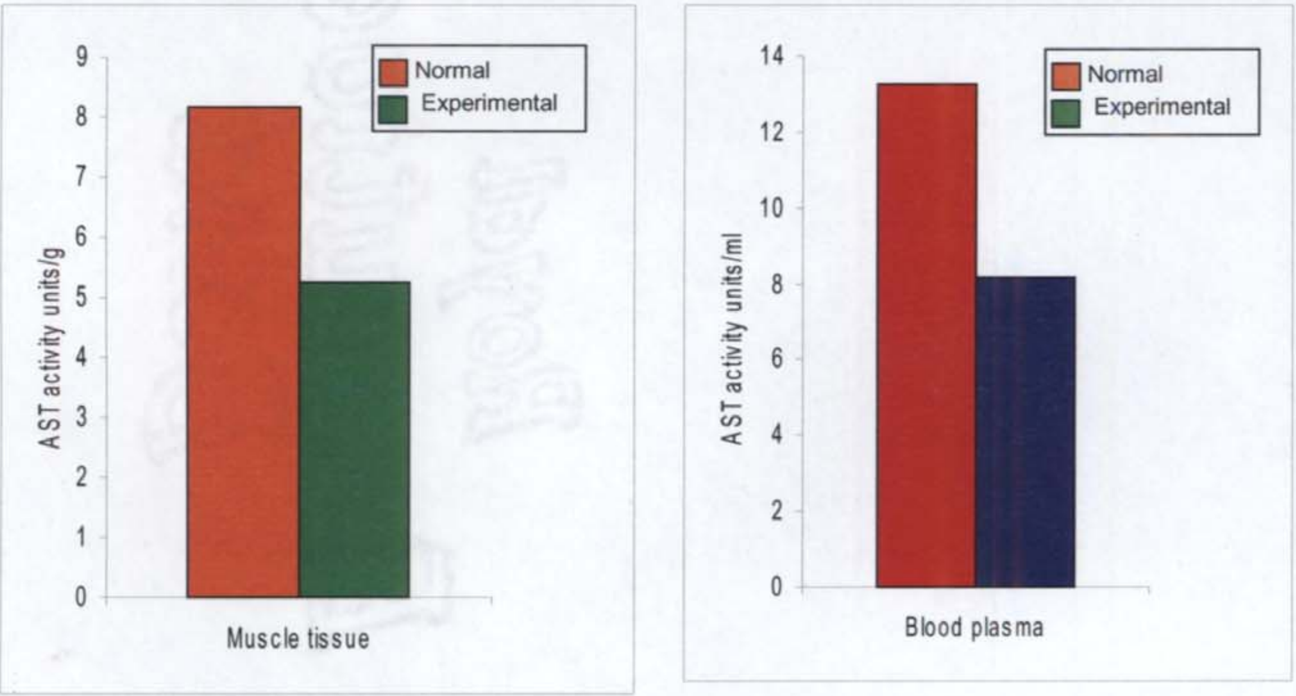


Figure 8. Aspartate amino transferase activity in muscle tissue and blood plasma in normal and experimental birds

Protease activity

The enzymatic activity of protease in the muscle tissue and blood plasma of both normal and experimental birds are presented in the table 9 and figure 9.

Table: 9. Protease activity in muscle tissue and blood plasma in normal and experimental birds

Experimental conditon	Protease activity	
	Muscle tissue, units/g	Blood plasma, units/ml
Normal	5.11±0.62	8.14±0.93
Experimental	13.44±2.15	10.95±1.66

The values are the means of five determinations with standard deviations.

There was a sharp increase in the activity of protease in the tissues of experimental birds compared to the normal birds. In the muscle tissue the activity was more than doubled whereas in the blood plasma the increase was only about 25%.

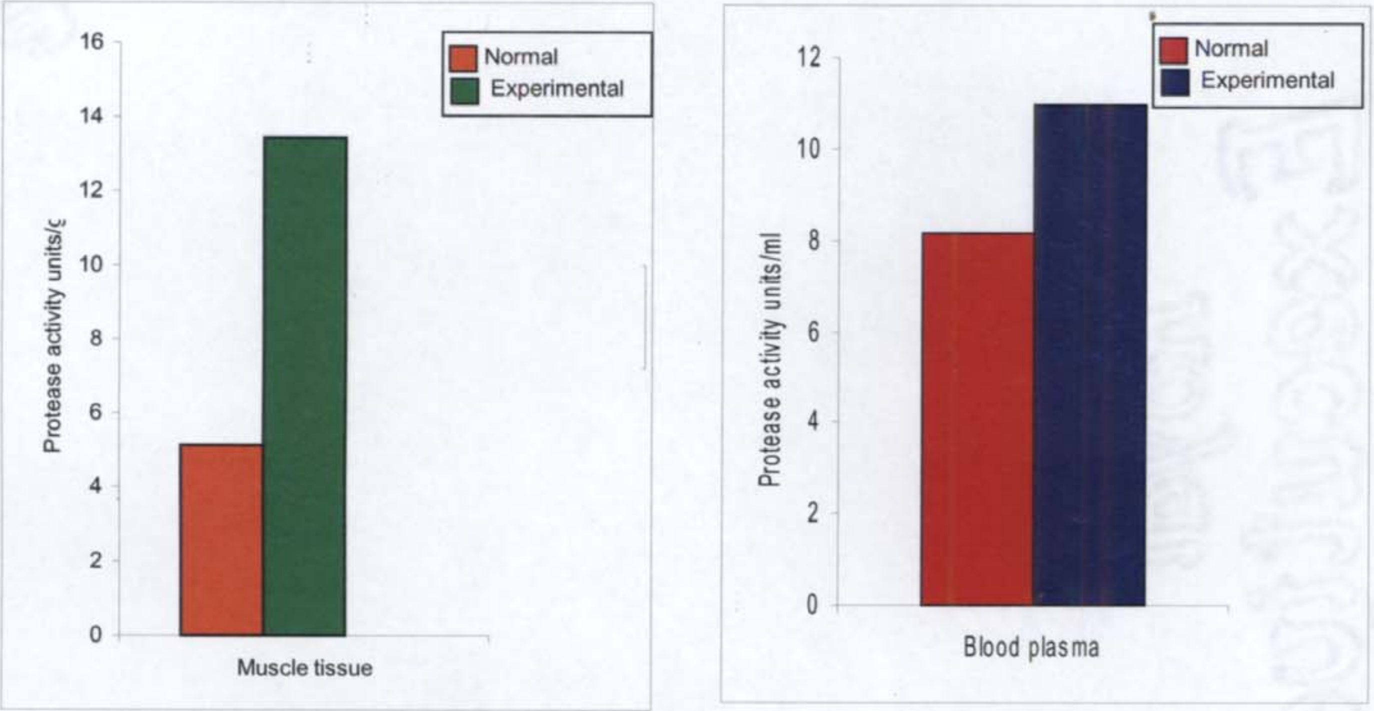


Figure 9. Protease activity in muscle tissue and blood plasma in normal and experimental birds

Total lipids

Total lipid concentrations in the muscle tissue and blood plasma of both normal and experimental birds are recorded in the table 10 and figure 10.

Table: 10. Total lipid concentrations in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Total lipid	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	10.1±0.31	19.2±1.22
Experimental	4.48±0.74	12.6±0.55

The values are the means of five determinations with standard deviations.

There was a conspicuous decline in the concentration of total lipids in the muscle tissue and blood plasma of experimental birds. The reduction of the total lipids in the muscle tissue of experimental birds was less than half of its normal value whereas its reduction in the blood plasma was about 37%.

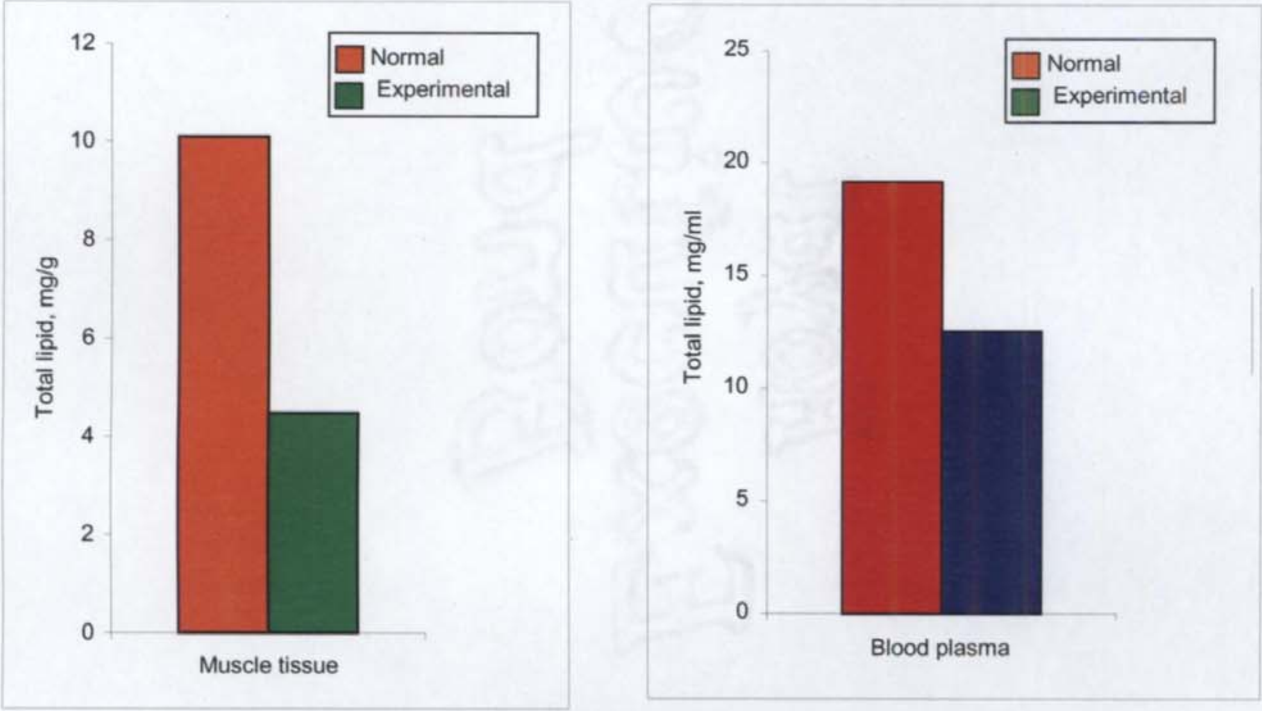


Figure 10. Total lipid concentrations in muscle tissue and blood plasma in normal and experimental birds

Total carbohydrates

Total carbohydrate concentrations in the muscle tissue and blood plasma of both normal and experimental birds are recorded in the table 11 and figure 11.

Table: 11. Total carbohydrate concentrations in muscle tissue and blood plasma in normal and experimental birds

Experimental condition	Total carbohydrate	
	Muscle tissue, mg/g	Blood plasma, mg/ml
Normal	6.90±0.92	10.83±1.63
Experimental	17.96±2.06	21.66±2.87

The values are the means of five determinations with standard deviations.

Concentrations of total carbohydrate value was increased in the experimental conditions compared to the normal conditions. The increase in the total carbohydrate in the experimental birds was 61% and 49% in the case of muscle tissue and blood plasma respectively.

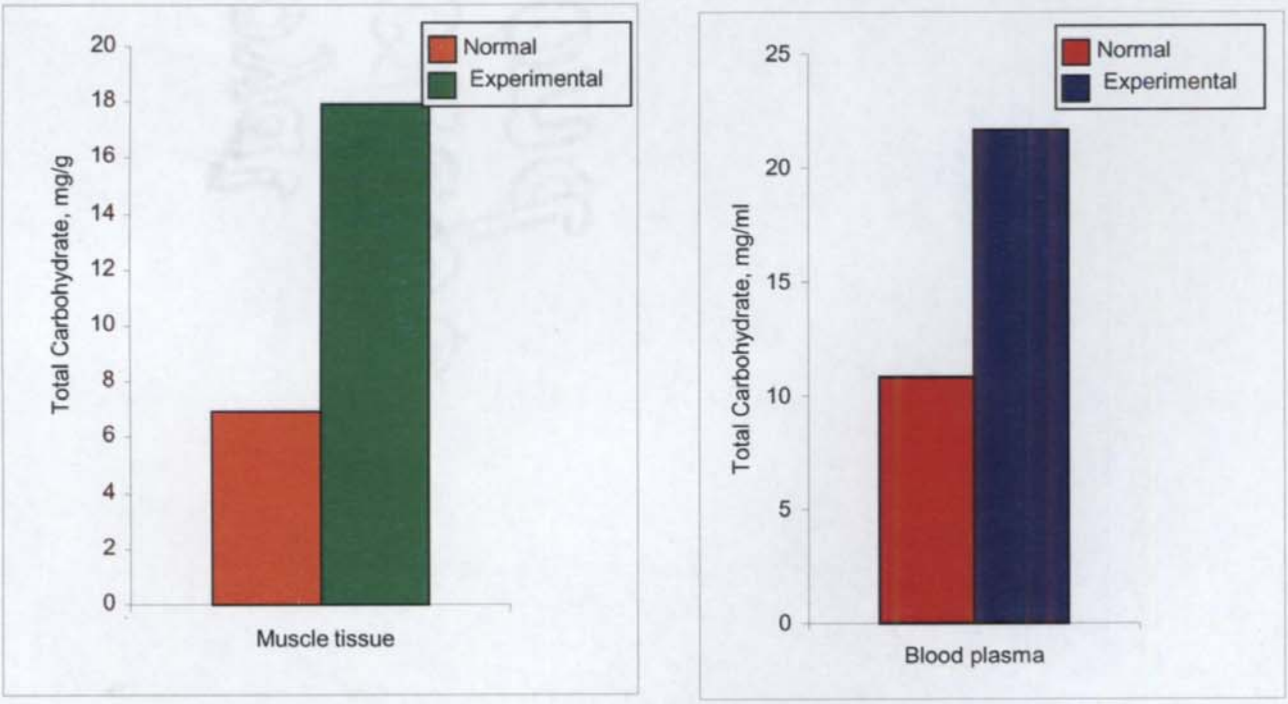


Figure 11. Total carbohydrate concentrations in muscle tissue and blood plasma in normal and experimental birds

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BIOCHEMICAL STUDIES ON BIRD FLIGHT

Thesis submitted to the University of Calicut
for the Degree of Doctor of Philosophy
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By

T.V. SHAJITHA

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, INDIA

2005

Discussion

71P

DISCUSSION

The energy requirements of avian flight have intrigued ornithologists for decades, particularly because birds are capable of flying that are spectacular in comparison with those of non-flying mammals, for example, some birds appear to fly at air speeds in excess of 15m/sec for tens of hours an endurance feat that no running mammal can match. The combination of high speed and endurance allows some birds to travel tens of thousands of kilometers per year – a distance that far exceeds those covered in a year by running mammals. Birds like Golden Plover flies over the Atlantic Ocean covering a distance of nearly 2400 miles without stopping for food or rest (George and Berger, 1966). The above facts point to a highly specialized biochemical mechanism of flight in birds. In the following discussion attempts have been made to evaluate the changes in the biochemical composition of the muscle tissue and blood plasma of the experimental bird on the excitation of its flight muscles.

Composition of tissues during normal and experimental conditions

Total proteins

Proteins are the chief organic constituents in the body concerned with the regulation of all biochemical events in the organism (Harper *et al.*, 1993). In the case of birds it is common knowledge that the sustained flights of birds are fuelled largely by previously accumulated fat stores. However protein is also catabolized during flight (Bauchinger and Biebach, 1998; Jenni and Jenni – Eiermann, 1998), and some of it is stored before flight bouts (Gauttier *et al.*, 1992; Lindstrom and Piersma, 1993; Marsh, 1984; Mc Landress and Ravelling, 1981; Piersma and Jukema, 1990). Because there is no storage form of protein (apart from a small amino acid pool), a change in the amount of body protein entails a structural or functional change. Variation in organ mass (which affects mainly its protein mass) is considered to be an expression of phenotypic flexibility (Piersma, 1998). Such flexibility is regarded as an adaptation, that allows animals to cope successfully with a wide range of conditions and life cycle events (Piersma and Lindstrom, 1997). The reduction in the amount of total proteins of the muscle and blood plasma of the experimental birds indicate that proteins are extensively utilized during continued muscle contraction.

In migrating birds, protein loss during long-distance flight affect predominantly the breast muscles, the digestive organs including the liver, and the skin (Akesson *et al.*, 1992; Battley *et al.*, 2000; Biebach, 1998). Before long flight bouts several species have been shown to enlarge their pectoral muscles (Davidson and Evans, 1988; Deriedzic *et al.*, 1993; Dietz *et al.*, 1999; Fry *et al.*, 1972; Lundgren *et al.*, 1995; Marsh, 1984; Mc Landress and Raveling, 1981). It may be noted that the rate of protein catabolism during fasting increases dramatically with regard to the pattern of lean mass variation, when current fat stores drop below a critical level (Cherel *et al.*, 1988; Cherel and Groscolas, 1999; Jenni and Jenni Eiermann, 1998) and that a similar pattern is very likely to occur during endurance flight (Jenni *et al.*, 2000).

If protein catabolism occurs predominantly in certain organs, it remains to be determined whether the reduction in organs is an advantage (Piersma and Lindstrom, 1997) or a means of minimizing overall damage (in terms of obtaining protein from temporarily less important organs). Regarding the breast muscle hypertrophy prior to migration may represent a compensation of the flight engine to cope with the larger body mass due to fat stores (Lundgren *et al.*, 1995; Marsh, 1984; Pennycuick, 1975; 1978; Piersma and Gill, 1998) and an adaptation to the expected protein loss during endurance flight or a protein reserve carried to the breeding grounds e.g. in arctic breeders (Davidson and Evans, 1988). Body mass decreases continuously during flight, so birds could minimize their total energy expenditure thereby reducing their breast muscle tissue.

Schwilch *et al.* (2002) investigated two aspects of protein loss during migratory flight. First they investigated to study the pattern of protein loss among organs in relation to current fat stores in long distance migrants, they measured the lean mass of organs in a series of birds with different fat stores in the sample from Ventotene island (that had just completed a long distance flight over the Sahara and the Mediterranean sea). Then they focused on protein loss from the breast muscles during long distance flights. They evaluated whether the reduction in breast muscle mass is a metabolically induced constraint or an adaptation to the decrease in body mass during flight.

According to Jenni – Eiermann *et al.* (2002) lipid stored in adipose tissue are the main fuel during long flights, but several studies have also shown that there is a certain amount of protein catabolism during flight. The proportion of protein contributing to energy expenditure is as low as in long-term fasting resting birds, slightly lower than in mammals during endurance locomotion.

Studies of Gannes *et al.* (2001) showed that after flight, blood plasma concentration of uric acid betahydroxybutyrate were elevated over control and per flight values, indicating elevated protein and lipid metabolism during flight. The studies conducted by Schwilch *et al.* (2002) shows that during long distance flights, birds catabolize not only fat but also protein, protein catabolism entails a structural or functional loss. After a long distance flight, birds showed a marked increase in net protein loss when fat stores were nearing depletion (analogous to the late phase of endurance fasting when the rate of protein catabolism is increased).

Studies of Bordel and Haase (2000) showed that in homing pigeons protein catabolism is increased during endurance flight. Studies of Dohm *et* *al.* (1982) also support the hypothesis that muscle protein breakdown and amino acid catabolism are increased by exercise. During long distance flight protein source present in the flight and leg muscles can be catabolised (Bauchinger *et al.*, 2001; Doherty *et al.*, 2004). A dip in the total proteins in the muscle and blood plasma on the continued excitation of the pigeon muscle observed in the present study, are in tune with the studies reported above.

Total free amino acids

The titre of amino acids in the muscle tissue and blood plasma can be used as a pointer in the evaluation of the dynamic metabolism of proteins and of the organism in total. A change in the amino acid pool will directly influence the protein turnover, i.e., the synthesis and degradation of proteins, and thus obviously reflect the physiological state of organism. The changes observed in the total free amino acids in the muscle tissue and blood plasma of normal and experimental conditions points to the dynamic protein turnover taking place during these conditions.

The actual picture of these dynamic changes can be obtained by evaluating the changes in the total content of free amino acids in the muscle tissue and blood plasma. The results of the present study revealed that in the excited muscles there was a conspicuous decline in the total proteins with a concomitant increase in the titre of total free amino acids and proteases. It is obvious that the sharp increase in the activity of protease during the excitation of the muscle provide a ready supply of free amino acids that can be used for gluconeogenesis. The results point to the importance of protein as a source of energy during flight in birds.

Levels of individual free amino acids

Glutamate

The concentration of glutamate was low in both experimental conditions of muscle tissue and blood plasma compared to normal conditions. Glutamate is an important amino acid in the synthesis of purines, pyramidines and NAD and in the transamination reactions which form the connecting link between the metabolism of carbohydrates and proteins (Lazar, 1983).

Proline

The amount of proline was higher in the experimental condition of muscle tissue but was low in blood plasma, compared to the normal conditions. Proline has a multiple function in the animal, as an energy source (Bursell, 1963, 1966; Bursell *et al.*, 1974; Sacktor, 1975) and as a precursor in the synthesis of structural proteins. Arginine is the precursor for the synthesis of proline. The conversion of arginine to proline results in the production of urea, which is an important adaptation in terrestrial organisms. The results of the present study do not provide any evidence to show that proline is used as an energy source during bird muscle contraction.

Lysine, Arginine

In the excited muscles there was a dip in the concentration of lysine with a sharp rise in its level in the blood plasma compared to normal conditions. In the case of arginine both tissues of the experimental birds recorded a striking increase in its level. The elevated levels of lysine and arginine may help the bird to maintain its acid-base balance and removing ammonium ion from the active metabolic pool during continued excitation of its muscle.

Glycine

The amount of glycine was more or less same in both muscle tissue and blood plasma of the experimental bird. Glycine belongs to the serine family of amino acids and is involved in the synthesis of structural proteins of animals (Seifter and Gallop, 1966).

Aspartate

There was a striking increase in the titre of aspartate in the experimental condition of muscle tissue on its continued excitation compared with normal conditions. The amount of blood plasma aspartate was more or less similar in both normal and experimental conditions. Aspartate is an important precursor in the synthesis of purines, pyramidins and structural proteins. Aspartate also, play an important role in transamination reactions and thus functioning as a connecting link between the metabolism of carbohydrates and proteins (Katunuma *et al.*, 1968). An elevated level of aspartate indicate gluconeogenesis and provide the much needed glucose for the muscles during excitation.

Threonine

The concentration of the threonine was more or less the same in both normal and experimental conditions of the muscles. However there was a decline in its concentration in the blood in experimental condition. Threonine can be converted to glycine and then to serine, which in tissue catabolised for liberating energy. Thus theronine involves in the formation of proteins and energy metabolism (Mehler, 1988).

Serine

The concentration of serine was low in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions. The biosynthesis of serine occurs via the phosphorylated pathway (Umbarger, 1978). Serine is involved in the synthesis of structural proteins of animals.

Valine

The amount of valine was doubled in the experimental condition of the muscle tissue but was low in blood plasma compared to normal conditions. Valine belongs to the pyruvate family. During degradation valine is transaminated to corresponding α-keto acids, which are then oxidatively decarboxylated to yield a derivative of CoA. Valine is involved in the energy metabolism and protein synthesis (Umbarger, 1978).

Alanine

The amount of alanine was higher in the experimental condition of the muscle tissue but was low in the blood plasma compared to normal conditions. Alanine is an important compound of the structural proteins and its increase indicates extensive degradation of muscle proteins. Alanine is also involved in gluconeogenesis via, transamination reactions. Alanine belongs to the pyruvate family of amino acids.

Phenylalanine and Tyrosine

The concentration of phenylalanine was slightly low in the experimental muscle but was increased three fold in the blood plasma compared to the normal. The amount of tyrosine was more or less the same in the muscle tissue both in the normal and experimental conditions, but showed a striking dip in the blood plasma of the experimental birds. Phenylalanine and tyrosine belongs to aromatic family of amino acids. Phenylalanine is converted to tyrosine (Stryer, 1988).

Histidine

Histidine was the second predominant free amino acid in the muscle tissue and blood plasma of the experimental bird. Its titre showed a significant dip in the tissues of the experimental birds indicating its extensive utilization during the excitation of the muscles. A high titre of histidine may help the animal in its acid-base balance. Histidine can be catabolized to glutamate. Its decarboxylation produces histamine (Stryer, 1988).

Taurine

Taurine is the most abundant free amino acid found in the tissues of pigeon. The concentration of taurine was higher in both experimental conditions compared to the normal conditions.

Taurine is biochemically an inert end product of methionine and cysteine metabolism, along with inorganic sulfate. Free taurine, has been found in millimolar concentrations, especially in the tissues that are excitable, and are rich in membranes that generate oxidants (Hishida and Nau, 1998). The role of taurine as a nutrient is to protect cell membranes by attenuating toxic compounds. Taurine is most abundant where reduced oxygen molecules are generated and where other potentially toxic substances, such as bile acids, retinoids, and xenobiotics are found. The chemical reactivity of the amino group allows taurine to perform this function, and it is probably the sulfonic acid end of the molecule that is critical in its performance (Charles *et al.*, 1986).

Like other amino acids, taurine behaves as an amphoteric electrolyte; however, some physicochemical differences in taurine are expected as a result of the presence of the sulfonate ion. Taurine possesses a more acidic acid function as well as a more acidic ammonium function than other amino acids (Thomas *et al.*, 1983). The capacity of amino acid dipole ions to form a metal complex is an important feature of their biological activity. Taurine forms less stable metal complexes with various transition metals than do other amino acids, as indicated by small stability constants.

Biosynthesis of taurine (Oja and Kontro, 1985) from precursor cysteine takes place in the following pathways: (a) oxidation of cysteine to 3-sulfinoalanine and then to cysteic acid, which is decarboylated to taurine; (b) oxidation of cysteine to 3-sulfinoalanine and decarboxylation of the latter to hypotaurine, which is oxidised to taurine; (c) reaction of cysteine, which is decarboxylated to phosphopantethein, from which cysteamine is split off and oxidised to hypotaurine, which is oxidised to taurine.

Role of taurine in membrane protection

There are now six lines of evidence suggesting that taurine plays an important role in the stabilization of membranes. 1. Hayes *et al.* (1975) demonstrate the disruption of the membranes of the photoreceptor cell in the taurine-deficient cat. This work has been extended to include effects on the tapetum lucidum of the taurine-deficient cat (Wen *et al.*, 1979) and on the cones of the photoreceptor layer of the taurine deficient monkey (Sturman *et al.*, 1984). 2. Taurine counteracts damage caused by external agents to skeletal muscle, intracellular membrane (Huxtable and Bressler, 1973) and heart muscle (Kramer *et al.*, 1981). 3. Taurine protects isolated rod outer segments from frog retina against structural damage induced by illumination and by oxidants (Cruz and Pasentes-Morales, 1983). 4. Taurine protects cultured cells against retinol-induced and ferrous sulfate-induced damage and/or swelling (Pasantes-Morales *et al.*, 1984; Pasantes-Morales *et al.*, 1985). 5. Taurine has an antioxidant effect in rabbit spermatozoa (Alvarez and Storey, 1984). This raises the possibility that the protective effects mentioned above might be mediated through an ability to reduce lipid peroxidation. 6. Taurine protects the hepatic cells against damage to the membrane by carbon-tetrachloride (Nakashima *et al.*, 1983).

The membrane stabilizing effect of taurine has been examined in cultured human lymphoblastoid cells (Pasantes-Morales *et al.*, 1984). Damage to the membrane results in increased permeability and transfer of water. Thus, taurine protects the cells from damage caused by iron-ascorbate, but it does not do so through a reduction in the extent of lipid peroxidation. Rather, the protective effect seems to be related to an action on permeability to ions and water.

These differences in taurin's protective effects may be related to differences in the reduced oxygen molecules involved. The oxidative effects may be exerted on components other than polyunsaturated fatty acids. Such oxidative effects would not be detected by the thiobarbituric reaction. It is possible also that the peroxidative effects and the nonperoxidative effects both result in permeability changes, which in turn, result in osmotic changes due to shifts in water and ions, with resulting cell swelling and damage (Van Gender and Barbeau, 1985). Whether the protective effect of taurine on membranes is direct or indirect remains to be determined.

Other evidence also suggests that the protective effect of taurine is mediated through an action on membrane permeability and ion transport. Taurine modifies calcium fluxes in sarcolemmal, synaptosomal and retinal subcellular fractions (Azari and Huxtable, 1980; Pasantes-Morales *et al.*, 1979; Pasantes-Morales and Gamboa, 1980). It induces changes in transmembrane potentials consistent with modifications in potassium permeability (Gruener *et al.*, 1975). It has been related to changes in sodium gradients in cultured cells (Kurzinger and Hamprecht, 1981) and to osmolar changes in marine invertebrates (Allen and Garrett, 1971).

Therefore, taurine seems to counteract a variety of agents that result in leaky membranes. Taurine may have a dual role in this regard. First, it may react with these deleterious agents via its amino group to detoxify them.

Second, it may also exert a direct protective effect in preventing the ionic and water shifts that result in cellular damage and death.

During the excitation of the muscles, maintenance of its membrane protection and permeability are critical especially when the muscle proteins are depleted. The abundance of taurine in the pigeon tissues can be correlated with its role in the membrane protection and permeability during exigenecies such as continued activation of muscles.

Role of taurine in detoxification

Lithocholic acid and its conjugates are known to induce intrahepatic cholestasis in a variety of experimental animals (Yousef *et al.*, 1981). Cholestasis results even through lithocholate is normally only a minor component of bile that undergoes sulfation, a process thought to reduce its potential toxicity. Administration of taurine (0.5%) in the drinking water one, two or five days before intravenous injection of lithocholic acid sulfate prevents the cholestasis and the morphological changes that result from administration of this is secondary bile acid. Taurine conjugates with certain xenobiotics. Not only are endogenously produced toxins, such as secondary bile acids, detoxified with taurine, but exogenous toxins can be conjugated as well. Taurine is a byproduct of the metabolism of the sulphur containing amino acids cysteine and methionine. Maintenance of a high titre of taurine in the bird tissues may help them to prevent the build-up of sulphur containing amino acid during continued excitation and thus play an active role in detoxification.

Glucose

The concentration of glucose was elevated in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions. Similar increase in the circulating levels of glucose, lactate, adrenaline and noradrenaline after a flight of 48 km lasting 60-80 minutes by homing pigeon have been observed by Viswanathan *et al.* (1987). It stimulated the release of glucagon which could account for the increase in plasma glucose. There was no change in plasma corticosterone concentrations. This is due to the fact that the birds were not under any serious stress during the flight. The increase in lactate has been attributed mainly to the activity of the white glycolytic fibres in the flight muscles. The increase in adrenaline and noradrenaline indicated increased sympathetic activity.

The increase in the glucose may also be explained in the context of the elevated levels of proteolysis, increased titre of free amino acids and the changing levels of transaminases. The free amino acids generated by proteolysis can be converted to glucose by gluconeogenesis.

Levels of urea

The level of urea was elevated in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions. The results are in tune with the decline in the titre of protein and elevated proteolysis observed in the bird on the continued excitation of its muscles. Bordel and Haase (2000) have observed that immediately after flight, plasma urea and uric acid levels of the homing pigeons were increased. They suggested that its protein catabolism is increased during endurance flight with a concomitant increase in the amount of urea. Urea is one of the chief end products of amino acid metabolism. The result apparently indicates the role of amino acids in the flight metabolism of birds.

Creatinine

The amount of creatinine showed an increase in the muscle tissue and blood plasma of the birds on continued excitation of its muscles. Creatine phosphate, the phosphorylated derivative of creatine found in vertebrate muscles, is a high-energy compound that can reversibly donate a phosphate group to ADP to form ATP. Creatine is synthesized from glycine and the guanidino group of arginine, plus a methyl group from s-adenosyl methionine. Creatine and phosphocreatine spontaneously cyclizize at a slow but constant rate to form creatinine, which is excreted through urine (Champa and Harvey, 1994). The turnover rate of creatinine has long been considerd as a pointer in the evaluation of the active protoplasmic mass in vertebrates. The elevated level of creatinine in the tissues of the experimental bird on excitation of its muscles correlated well with the above view.

Aminotransferases activity

Compared to mammals there are very little information on aminotransferases in aves which are known to play a key role in the intermediary metabolism of amino acids. In the present study the level of AST and ALT activity in the pigeon showed variation during continuous muscle contraction. The amount of aspartate aminotransferase was low in the experimental conditions of muscle tissue and blood plasma compared to normal conditions. The amount of alanine aminotransferase was just opposite to the above case as its value was higher in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions.

The important physiological functions of aminotransferences are the maintenance of the amino acid pool at a proper level for protein synthesis (Meister, 1965), the supply of metabolites for energy metabolism (Sacktor, 1974) and the catalysis of interactions between protein and carbohydrate metabolism. Starvation can induce a high aminotransferase activity in the mammalian liver. This condition is linked to gluconeogenesis for substituting the carbohydrates (Katunuma *et al.*, 1968).

The protein synthesis requires a balanced amino acid pool and transamination is one of the chief mechanisms, which functions as a regulator for this (Reddy *et al.*, 1991). Higher transaminase implies enhanced mobilization of free amino acids into transamination activities. The alanine aminotransferase activity forms a general index of amino acid break down and aspartate aminotransferase marks the mobilization of amino acids into gluconeogenesis (Adibi, 1968; Davidson and Longslow, 1975).

Protease activity

Activity of protease showed a striking increase in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions. The results are indicative of the proteolysis of the stored muscle proteins during continued excitation of the muscles. The proteolysis yields amino acids which can be used for generating glucose via gluconeogenesis and this provide the much needed energy resource during the flight metabolism.

Total Lipids

Lipids stored in adipose tissue are the main fuel during long flights (Jenni and Jenni-Eiermann, 1998). The delivery of lipids from adipose tissue to the working muscles is facilitated in various ways. Fatty acids are insoluble in the aqueous medium of blood and cells and need to be bound to a carrier to be transported. The re-esterification of fatty acids in the liver and delivery to the flight muscles as very-low-density lipoproteins may be a means of circumventing the constraints in the blood stream of fatty acid supply to the muscles in small passerines (Jenni-Eiermann and Jenni, 1992). This results in high concentration of triglycerides in the plasma. The transport of fatty acids within muscle cells is likely to be optimized by the highest concentration of fatty acid binding protein found in vertebrates (Guglielmo *et al.*, 1998).

The concentration of total lipids showed a marked decline in the experimental conditions of muscle tissue and blood plasma compared to the normal conditions.

In homing pigeons, plasma metabolite concentrations and respiratory quotient show that a gradual switch to a lipid based energy delivery occurs during 30 minute to 2 h of flight (Bordel and Haase, 1993; George *et al.*, 1989; John *et al.*, 1988). However there are no detailed studies on true migrants with known flight durations. The types of fuel used during endurance flight, i.e., the proportion of lipids and proteins may vary among and within individuals depends on body mass, flight style and ambient conditions. Protein from wet tissue is 8-10 times less energy dense than lipids in adipose tissue (Jenni and Jenni – Eiermann 1999).

According to Jenni – Eiermann *et al.* (2002) most of the energy needed for prolonged exercise in migrating birds is provided by the oxidation of lipids, which are stored as triglycerides mainly in adipose tissue. In the plasma of flying knots, they measured increased levels of free fatty acid and glycerol in comparison with resting birds fasting for the same period, indicating increased hydrolysis of triglycerides from adipose tissue. This finding is in agreement with all studies of exercising birds, e.g. domestic fowl running on a tred mill (Brackenbury and El-sayed, 1984), flying pigeons (Bordel and Haase, 1993; George *et al.*, 1989; John *et al.*, 1988; Schwilch *et al.*, 1996; Vallyathan and George, 1969) and small passerines (Jenni – Eiermann and Jenni, 1991) as well as studies on exercising mammals and humans (Keul, 1975; Paul, 1975). The results of the present study are in tune with the above findings.

Total carbohydrates

The concentration of total carbohydrate was increased in the experimental conditions of the muscle tissue and blood plasma compared to the normal conditions. In the case of birds the immediate source of energy for muscle contraction appears to be ATP, the ultimate source is carbohydrate or fat. The initiation of flight require a highly efficient oxidative metabolism which is fueled by carbohydrates (George, 1962). The elevated levels of carbohydrates on the continued excitation of the muscles may be viewed in the context of the elevated levels of free amino acids and glucose in the same period. The glucose generated through gluconeogenesis may be utilized for energy for flight.

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BIOCHEMICAL STUDIES ON BIRD FLIGHT

Thesis submitted to the University of Calicut
for the Degree of Doctor of Philosophy
under the Faculty of Science

By

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2005

General Discussion and Conclusions

GENERAL DISCUSSION AND CONCLUSIONS

The present investigation concerned with the flight metabolism of birds and to evaluate the energy resources during flight. During migration or sustained flights, birds are fuelled mainly by previously accumulated fat stores. For efficient oxidative metabolism, in any organ or tissue a copious blood supply is an essential prerequisite. The breast muscles of birds indulging in sustained activity are an example to illustrate it. High lipolytic activity should facilitate the release and movement of free fatty acids for oxidation. However, it should be mentioned that if the products of fat catabolism are produced a rate faster than the capacity of the system to oxidize them, the acetic acid residue condense to form ketone bodies. It is necessary that the avian body should possess adequate mechanism for the prevention of ketone body accumulations as well as for oxidizing them if formed (George and Berger, 1966).

The reactions of ketone body formation occur in the matrix of liver mitochondria. Acetone, acetoacetate and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver (the extrahepatic tissues), where they are oxidised in the citric acid cycle to provide much of the energy required by tissues such as skeletal and heart muscle and renal cortex. The brain, which preferentially uses glucose as a fuel, can adapt to the use of acetoacetate or D-β-hydroxybutyrate under starvation conditions, when glucose is unavailable. The production and export of ketone bodies from the liver to extrahepatic tissues allows continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle (Olpin, 2004).

In extra hepatic tissues, D-β-hydroxybutyrate is oxidized to acetoacetae by D-β-hydroxybutyrate dehydrogenase. Acetoacetate is activated to its CoA ester by tranfer of CoA from succinyl-CoA, an intermedaite of the citric acid cycle, in a reaction catalyzed by β-ketoacyl-CoA transferase. The acetoacetyl-CoA is then cleaved by thiolase to yield two acetyl-CoAs, which enter the citric acid cycle (Hasselbalch *et al.*, 1994). Similar mechanisms of ketone body oxidations are possible in birds. This can form a major preventive measure of the accumulation of ketone bodies in birds during prolonged flight.

Taurine was the most abundant amino acid in the muscle tissue and blood plasma of the experimental birds. Taurine may have a dual role in membrane protection: it may react with deleterious agents via its amino group to detoxify them and it may exert a direct protective effect in preventing the ionic water shifts that results in cellular damage and death. The role of taurine in membrane protection is discussed elsewhere. Taurine also act as a general detoxifier, eliminating cholates, removing xenobiotics and scavenging chlorine oxidants (Pasantes-Morales, 1984). Taurine may thereby specifically protect cells from self-destruction during processes that generate oxidants (Pasantes-Morales, 1985).

The role of taurine in fat metabolism and its utilization is also well known (Emmert, 1996). Most importantly taurine helps to prevent the accumulation of ketone bodies. The most abundant bile acids in humans are cholic acid and chenodeoxy cholic acid. Bile acids are steroid derivatives with detergent properties, which emulsify dietary lipids in the intestine and thereby promote fat digestion and absorption. The cholic acid and chenodeoxycholic acids are usually conjugated in amide linkage with the amino acid glycine or taurine, giving compounds called bile salts. The abundance of taurine in the tissues of the pigeon observed in the present study points to its role in detoxication mechanisms during sustained muscle contraction.

The cholic acid conjugates with taurine and glycine are called taurocholate and glycocholate, respectively. The bile salts are stored in the gall bladder, and released into the small intestine after ingestion of a fatty meal. These amphipathic compounds act as biological detergents, converting dietary fats into mixed micelles of bile salts and triacylglyceroles. Further, triacylglycerol can be converted to glucose through gluconeogenesis (Van Coster *et al.*, 1991). The de novo synthesis of glucose facilitates the birds to meet its energy demand during flight. The role of taurine in the flight metabolism of birds need to be investigated in detail to make conclusions on the above.

Biochemical studies on birds showed that avian metabolism is similar to that of other animals with the exception of specialized pathways suited to preventing the accumulation of ketone bodies (Morris, 2005). As in other animals glucose appears to be a major immediate source. For avian cells however extensive fatty acid utilization and gluconeogenesis are other alterative energy sources during exigencies. It had been observed that during endurance flight up to 20% of the initial body mass is used as fuel (Odum, 1958). The results of the present study indicate that lipids and proteins are considerably reduced in muscles and blood plasma and an increase in glucose concentration. An elevated level of gluconeogenesis through proteolysis during flight may reduce ketogenesis due to the excessive use of fatty acids as fuel for flight.

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