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# A Biochemical Study of Fasting, Subfeeding, and Recovery Processes in Yellow-Legged Gulls

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## ABSTRACT

An investigation of the effects of fasting, subfeeding, and refeeding on plasma biochemistry was carried out on 22 captive yellow-legged gulls *Larus cachinnans* Pallas. These birds showed the same fasting endurance model described in other species, but with an important decrease in glucose plasma concentration and very great differences between individuals when reaching the deterioration limit, suggesting a moderate physiological adaptation to long periods of fasting. A different model was proposed in subfed gulls in relation to fasted gulls, based on lipid and protein use, which could be reflected by changes in nitrogen wastes and triglyceride levels in this experiment. Thus, the subfed gulls might use protein directly from the diet as an energy source, thereby reducing the use of fat stores. The gulls quickly recovered body mass during the refeeding period, but while some plasma substances quickly reached their initial values, others showed many changes before the end of the experiment, which could reflect a process of metabolic restabilization. These results contribute to a better knowledge of fasting, subfeeding, and refeeding processes in birds and can be added to a recent study about fasting in gulls.

## Introduction

Many studies have been developed on the physiological response of birds when enduring food restriction. These works suggest three different phases based on changes in body weight and plasma biochemistry (see Fig. 1), which we will name the "classic model" (e.g., Le Maho et al. 1981; Boismenu et al. 1992; Handrich et al. 1993*b*). In a first phase (phase 1), body weight shows an important reduction in a short interval of time, whereas the second phase (phase 2) presents a slow and stable daily weight descent, during a more or less long period, depending on the species. The final phase reveals a quick and strong increase in body-mass loss to reach a critical level close to death (phase 3). The fasting-adapted species relies on fat as the primary energy source during fasting periods, spares protein, and relies primarily on protein only when fat reserves are depleted (see, e.g., Cherel et al. 1988*a*). Protein is spared due to its key role in body structure and muscle function and as enzymes (Felig 1979; Castellini and Rea 1992). In this model, plasma levels of residuals from protein catabolism (urea and uric acid) decrease during phase 1, maintain a stable low concentration or a slow increment in phase 2, and rise suddenly to reach the highest levels during phase 3 (use of structural proteins as energy source), which might indicate the bird's death. Changes in excretion of these nitrogen residuals are in fact parallel to those observed in daily body-mass change. However, in birds, as in mammals, by far the largest reservoir of body fuel is in the form of fat, stored as triglycerides (Cherel et al. 1988*a*; Castellini and Rea 1992). In the classic model, free fatty acids and ketone bodies increased in blood plasma in phase 1 as a consequence of triglycerides breaking down, which reflects their use as an energy source; they maintain high concentrations in phase 2 and abruptly decrease in phase 3 (exhaustion of fat stores). Plasma triglycerides steadily decrease during fasting (Fig. 1), though they were not described on the basis of the classic model (Jenni-Eiermann and Jenni 1994). Finally, glucose maintains its concentration, only decreasing in phase 3 (Cherel et al. 1988*b*; Boismenu et al. 1992), reflecting its importance in birds' metabolism. In fact, this carbohydrate is a critical fuel for the central nervous system, and its circulating concentration is tightly regulated (Castellini and Rea 1992).

On the other hand, the physiological means for supporting absolute fasting could be different from a limited food restriction, a phenomenon probably more extended in species with very diverse food resources, such as gulls (Cramp and Simmons 1983; Munilla 1997). Comparison between the effects of a reduced diet in relation to the effects of fasting in wild species of birds has not been documented in bibliographies, as far as we know. This kind of study might help develop understanding of the usual physiological state of many individuals during subfeeding periods.

In contrast with the studies on fasting, the refeeding period

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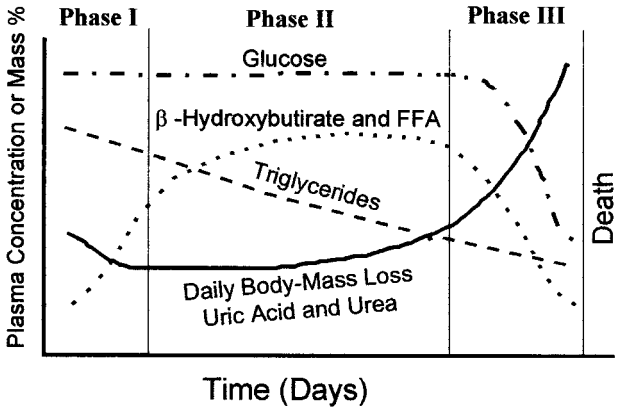


Figure 1. Ideal pattern of changes in plasma composition and body mass during fasting in bird species (classic model). Daily body-mass loss is the body-weight proportion with respect to the previous fasting day (the scale on *Y*-axis is only illustrative).

in wild bird species has been poorly studied. Handrich et al. (1993*a*) proposed a model consisting of two phases. The first one is the recovery of initial body weight and the restoration of prefasting metabolic rates. The second phase is a period of steady body mass and metabolism. The García-Rodríguez et al. (1987) study showed a slow recovery of body weight in buzzards (*Buteo buteo* Linnaeus) throughout the refeeding period, while plasma uric acid levels declined abruptly at the beginning but slowly reached the original concentrations of fed birds, in agreement with the second phase proposed by Handrich et al. (1993*a*). More research on this process is needed.

In summary, this study has three objectives: (1) to know whether a seabird, the yellow-legged gull *Larus cachinnans* Pallas, uses the above-cited classic model of resource allocation during starvation, as shown in other bird species (thus, we will analyze differences with respect to the described pattern; Fig. 1). Moreover, (2) this species was chosen in order to compare changes during periods of moderate food restriction with periods of absolute fasting. Therefore, we might obtain a more realistic approach to some bird life histories. Finally, (3) we also studied the recovery process in the yellow-legged gull. With these three objectives, we analyzed 12 plasma parameters, representatives of proteins and their catabolic residuals (total protein, urea, and uric acid), fats such as triglycerides and cholesterol, carbohydrates such as glucose, and some enzymes and ions.

## Material and Methods

### *Experimental Procedure*

On February 28, 1999 (2 mo before the laying date in the colony), we captured 22 adult yellow-legged gulls on a refuse dump close to the city of Porriño (Pontevedra, Spain). These birds were transported to the wildlife recovery center La Cañada de los Pájaros (Huelva, Spain) and were housed in individual cages (4 × 4 × 4 m). The study was performed with the permission of the appropriate authorities, avoiding any damage to the birds. The gulls were divided into three groups, and the sex ratio was balanced. Sex was determined through the PCR amplification of CHD gene fragment sequences following Griffiths et al. (1998). In this way, nine individuals formed the fasting group (four males, five females), nine formed the restricted group (four males, five females), and the last four birds constituted the control group (two males, two females). For 2 wk, sardines (*Sardina pilchardus* Walbaum) were provided ad lib. Fish is present in 32% of the pellets in the original population of the experimental birds (sardines included; Munilla 1997), and its biomass proportion might be even higher. After this, from day 0 of the experiment the fasting group remained without food, the restricted group was fed one-third of the mean daily intake (calculated individually for each gull during the previous 2 wk), and the control group remained with ad lib. food. This interval was called the deterioration period. All birds had water ad lib. during the experiment. Variable total body-mass loss was defined as the proportion of body-mass loss regarding weight at the beginning of the experiment. The return to feeding (recovery period) was planned when birds would reach phase 3 of the classic model described in other species (i.e., Boismenu et al. 1992), but since there was no previous information about critical levels of body-mass change or biochemical parameters in this species, an a priori limit of total body-mass loss was fixed at 25% to start refeeding the birds. This limit was estimated conservatively from the proportion of total body-mass loss of three ill individuals (captured in previous years), which was calculated from the expected body weights regarding their body size in the original population (C. Alonso-Alvarez and M. Ferrer, unpublished observations). Nevertheless, after 8 d, three gulls from the fasting group died on the same day, without symptoms, and their lesser total body-mass loss the day before (15%) was finally established as the final limit before placing the gulls in the recovery period, refeeding them with food ad lib. Another two birds, one from the fasting group and the other from the restricted group, were retired after phase 3 because of a clear risk of death (inability to walk), and they were treated with glucosaline serum and vitamins so that they could recover their initial healthy state. The experiment finished when all the birds reached the confidence limits of total body-mass loss in relation to capture. After this, the gulls were set free in the original capture location.

### *Blood Extraction and Weighing Procedures*

Blood samples were taken from the humeral vein (2.5 mL) every 2 d throughout the experiment, always before feeding, at the middle of the day (1100–1500 hours) to avoid any variation in blood chemicals caused by the circadian rhythm (Ferrer

1993). Winged infusion sets (Valu-Set, Becton Dickinson, Sandy, Utah) were used to prevent damage to the veins, applying them on alternate wings each time. Blood sampling was done immediately after capture. Lithium-heparin was employed as anticoagulant, and the samples were stored between 0° and 4°C until they were carried to the laboratory a few hours after collection. Plasma was separated by centrifugation (550 *g* for 10 min) and was stored in a freezer for 1 d until the analysis. The gulls were weighed after blood collection with a dynamometer (Pesola; accuracy ±5 g). Daily body-mass loss represented the change in body weight with respect to the day before sampling, allowing us to see small changes from day to day.

### *Tested Parameters*

Twelve biochemical components of blood were measured using a spectrophotometer (Hitachi 747, Tokyo, Japan) and commercial kits (Boehringer-Mannheim Biochemica, Mannheim, Germany). The analyzed biochemical parameters were (abbreviations and methods indicated in parentheses): urea (UREA; urease method), uric acid (URIC; uricase method), triglycerides (TRIG; enzymatic method that includes amounts of free glycerol), total protein (TP; biuret reaction), creatinine (CREA; kinetic Jaffé reaction), inorganic phosphorus (iP; molybdenum blue reaction), calcium (Ca; cresolphtalein complexone reaction), magnesium (Mg; blue xilidil reaction), glucose (GLUC; hexocinase method), cholesterol (CHOL; cholesterol esterase), amylase (AMY; maltoheptaose reaction), and alkaline phosphatase (AP; paranitrophenyl-phosphate method).

### *Data Analysis*

Mean values of parameters were tested for differences between groups on the same day or in the same mass-loss rank by the Mann-Whitney *U*-test for independent samples. Within-group variations were tested with Wilcoxon matched pairs signed-ranks test. These nonparametric tests were used as a precaution since, as a result of small sample sizes in some analyses, normal distribution could not be ascertained for all parameters. The experiment effects were examined with repeated-measures ANOVA, where the treatment (fasting or subfeeding) was used as a factor (between-subject effect) and the samples obtained from the same bird throughout the experiment were used as repeated measures (within-subject effect). Moreover, repeated-measures ANOVA was used to analyze changes in mass or biochemical parameters in each group separately. A general linear model of variance analysis was developed in order to determine the influence of the treatment (group as fixed factor) and the influence of proportion of body-mass loss (total body-mass loss as covariable) in each biochemical parameter (dependent variable) throughout the experiment, using the individual as a random factor in order to avoid pseudoreplication. All tests were performed with SPSS software (Norusis 1993).

## Results

### *Initial and Final Values in the Deterioration Period*

Body mass (mean ± SE; males: 898.9 ± 24.2 g; females: 733.1 ± 7.9 g) and plasma biochemical values were measured the first day of the experiment in all birds. That day, there were no differences among the three groups or between sexes (Mann-Whitney: $P > 0.05$ in all parameters). The four birds from the control group did not show significant variations in total body-mass loss (repeated-measures ANOVA: $F_{10,30} = 1.07$, $P = 0.41$) and plasma biochemical traits (always $P > 0.05$) throughout the experiment and are not used in the rest of the statistical analyses. There were many significant differences (Wilcoxon: $P < 0.05$) in plasma concentrations between the first day and the last day of the deterioration period in gulls suffering fasting or food restriction (see Table 1). Urea, uric acid, cholesterol, glucose, and alkaline phosphatase changed in both groups. There were no significant differences between these two groups the last day of the deterioration period regarding all the parameters, but inorganic phosphorus, calcium, and magnesium showed a tendency toward higher values in the fasting group (Mann-Whitney: $P < 0.12$).

### *Weight and Biochemical Changes with Respect to the Classic Model*

In order to explain the changes in body mass throughout the deterioration period, we analyzed daily body-mass loss and total body-mass loss during the fasting phases according to the classic model (Fig. 2). For both variables, data of the first four sampling days from the beginning and, separately, data of the last four sampling days to reach the final limit of the deterioration period were analyzed ($n = 9$ in each group) in order to equilibrate the sample size between the groups. The sample size on some days was not equilibrated because of the highly variable number of days to reach the fixed deterioration limit among individuals (fasting group: 8–12 d; restricted group: 10–18 d).

Daily body-mass loss did not show significant within-subject differences in the first four sampling days (repeated-measures ANOVA: $F_{3,48} = 1.41$, $P = 0.25$), but it did show such a difference in the last 4 d ($F_{3,48} = 15.77$, $P < 0.001$). The differences between groups in the first four measurements and in the last 4 d (Fig. 2) showed a tendency to statistical significance ($F_{1,16} = 3.07$, $P = 0.09$; and $F_{1,16} = 3.47$, $P = 0.08$, respectively), showing lower values in the restricted group (Fig. 2). A descent in daily body-mass loss between the second and the fourth day (proposed phase 1) were not significant in either group (Wilcoxon: $Z = 0.77$, $P = 0.44$ in both groups). Daily body-mass loss measurements in the last day of the deterioration period (in proposed phase 3) were higher in the fasted

Table 1: Mean values (±SE) of plasma parameters the first and the last day of the deterioration period, differentiated by groups

| | Fasting Group | | Restricted Group | |
|---|---|---|---|---|
| Biochemicals | First Day | Last Day | First Day | Last Day |
| Urea (mg/dL) | 5.78 (.22) | 13.22 (1.63)* | 5.33 (.33) | 11.67 (1.54)** |
| Uric acid (mg/dL) | 9.97 (1.05) | 20.32 (2.93)* | 10.51 (1.98) | 18.17 (2.49)* |
| Total protein (g/dL) | 3.35 (.36) | 3.05 (.46) | 3.17 (.19) | 2.22 (.28)** |
| Triglycerides (mg/dL) | 75.56 (10.34) | 53.56 (9.90) | 73.67 (4.92) | 32.22 (4.42)** |
| Cholesterol (mg/dL) | 324.6 (35.4) | 201.9 (24.9)* | 375.6 (19.7) | 181.6 (15.5)** |
| Glucose (mg/dL) | 365.8 (15.9) | 267.8 (11.9)** | 325.8 (9.49) | 282.4 (9.0)* |
| Amylase (U/L) | 815.4 (62.7) | 653.9 (22.4) | 927.6 (88.3) | 890.1 (115.3) |
| Creatinine (mg/dL) | .26 (.003) | .22 (.003) | .26 (.002) | .17 (.002)** |
| AP (U/L) | 230 (52.2) | 99.56 (34.1)* | 223.2 (57) | 172.9 (44.1)* |
| Pi (mg/dL) | 3.28 (.25) | 4.75 (.63)* | 3.18 (.16) | 3.57 (.30) |
| Ca (mg/dL) | 8.73 (.50) | 8.25 (.54) | 8.69 (.23) | 7.17 (24)** |
| Mg (mg/dL) | 2.23 (.006) | 2.26 (.13) | 2.22 (.008) | 2.01 (.005) |

Note. Wilcoxon matched pairs signed-ranks test; $n = 9$ in each group. There are no differences among groups on the first ($P > 0.1$) or the last day ($P > 0.05$).

* $P < 0.05$.

** $P < 0.01$.

gulls than in the restricted gulls (Mann-Whitney: $Z = 1.99$, $P = 0.047$; see Fig. 2).

Concerning the biochemical parameters, we focused on uric acid and triglycerides as representatives of nitrogen residuals and fat use, respectively, synchronizing newly recorded data with respect to the first and the last day of the deterioration period (in Fig. 3, backward from last day). In the fasting gulls, uric acid increased in the first 4 d of the sampling and in the last four (repeated-measures ANOVA: $F_{3,24} = 7.21$, $P = 0.001$; and $F_{3,24} = 4.48$, $P = 0.012$, respectively). In the same group, triglycerides increased in the first four measures ($F_{3,24} = 6.66$, $P < 0.01$) and decreased in the last four ($F_{3,24} = 4.03$, $P < 0.05$). Differences between groups were detected in the last 4 d of the deterioration period (uric acid: $F_{1,16} = 8.24$, $P = 0.01$; triglycerides: $F_{1,16} = 11.23$, $P < 0.01$), but they were not significant in the first 4 d (uric acid: $F_{1,16} = 4.29$, $P = 0.06$; triglycerides: $F_{1,16} = 2.25$, $P = 0.16$). When we observed the deterioration period as a whole (see Table 2, "Group" column), we observed significant differences in triglycerides but not in uric acid, although it was close to statistical significance ($P = 0.07$; see also Fig. 5).

### *Weight Changes in the Recovery Period*

Changes in total body-mass loss during the recovery period were used to explain the return of our gulls to the initial body weight (Fig. 4). The values at last day of the deterioration period were significantly higher than the values at the first sampling day of the recovery period (Wilcoxon; fasting group: $Z = 2.02$, $P = 0.043$; restricted group: $Z = 2.52$, $P = 0.012$). Thus, after only 2 d of refeeding, the birds recovered a great part of mass they had lost, without differences between both groups (mean ± SE; fasting group: 49.3% ± 10.5%; restricted group: 69.9% ± 6.17%; Mann-Whitney: $Z = 1.71$, $P = 0.24$). There were not significant differences between groups throughout the recovery period (repeated-measures ANOVA: $F_{1,11} = 0.04$, $P = 0.86$). However, there was a significant decrease in total body-mass loss in the fasting group (within-subject effect: $F_{4,24} = 7.90$, $P = 0.001$) but not in the restricted group ($F_{4,39} = 1.77$, $P = 0.16$). Nevertheless, only a nonsignificant tendency to higher values of mass loss in the restricted group in the last day was detected (Mann-Whitney: $Z = 1.70$, $P = 0.09$; Fig. 4).

### *Changes in Biochemical Variables throughout the Experiment*

In this study we confronted two problems in the interpretation of data. The first one was the individual differences in the number of days to attain the deterioration limit (commented on above), which prevents changes from being analyzed with respect to a chronological order. The second problem was the obvious differences in total body-mass loss level between the groups in the same day, promoted by the effect of the treatment. This issue prevents between-group comparisons. With the aim of avoiding these problems, the changes were analyzed using a general linear model (Table 2), which allowed testing of the linear relationship of each biochemical trait with the proportion of total body-mass loss, but not with time.

In addition, we analyzed data by means of total body-mass loss ranks (Fig. 5), which allowed comparison of the plasma levels among the groups when the birds were in a similar body-mass proportion. Figure 5 included in the deterioration period

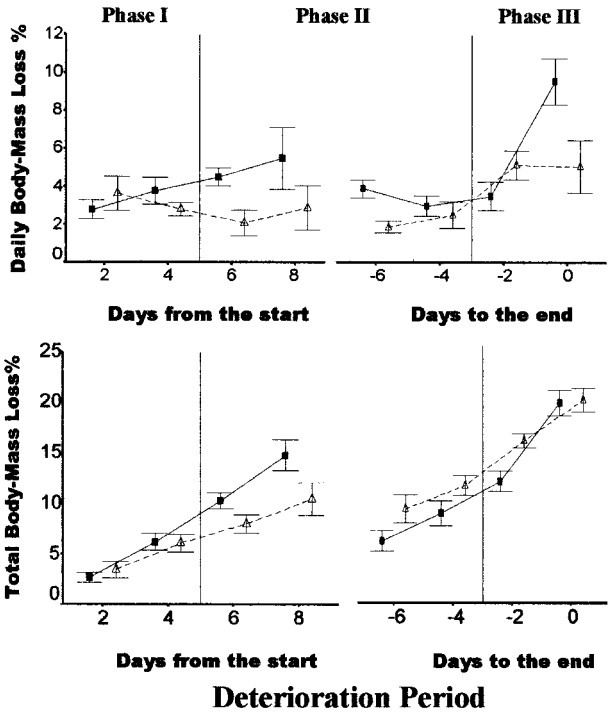


Figure 2. Changes in daily body-mass loss (body-weight proportion with respect to the previous sampling; *top*) and total body-mass loss (body-weight proportion in relation to day 0 of the experiment; *bottom*) throughout the deterioration period (food restriction). Individual data synchronized, starting forward from the first or backward from the last day of this period. Solid squares and continuous lines represent gulls in the fasting group, and open triangles and dotted lines represent the restricted gulls group. Proposed phases marked over graphics. (Means ± SE; $n = 9$, both groups.)

15%–25% ranks as a consequence of the sampling interval. Thus, birds could increase total body-mass loss from a value below 15% limit to 20%–25% rank after 2 d. In those gulls that reached the higher total body-mass loss rank (21%–25%; see Fig. 5), total protein, creatinine, inorganic phosphorus, calcium, and magnesium levels showed differences between groups (Mann-Whitney: total protein: $Z = 1.92$, $P = 0.055$; creatinine: $Z = 2.57$, $P = 0.010$; inorganic phosphorus: $Z = 1.71$, $P = 0.088$; calcium: $Z = 2.56$, $P = 0.011$; magnesium: $Z = 2.56$, $P = 0.011$; $n = 5$ in both groups), always with higher values in the fasting group.

Cholesterol, urea, and uric acid were the variables most strongly related to total body-mass loss during the deterioration period (see Table 2). The effects of the treatment in this period were significant in triglycerides (cited above), creatinine, and amylase concentrations (see Fig. 5). In the recovery period, calcium, total protein, creatinine, magnesium, cholesterol, and urea were the parameters related to total body-mass loss, whereas the rest of the variables were not affected by the changes in body mass. In this period, only urea and triglyceride concentrations significantly differed between the treatments.

## Discussion

### *Fasting Model*

One of the objectives of this study was to test whether the changes during fasting in yellow-legged gulls could be adjusted to the classic model with three periods (see Fig. 1) used by different authors (e.g., Cherel and Le Maho 1985; Boismenu et al. 1992; Handrich et al. 1993*b*). In this sense, daily body-mass loss did not diminish during the first days (called phase 1 in the classic model). This disagreement with other studies could be a consequence of an insufficient accuracy of measurement (±5 g). Alternatively, the sampling interval (2 d) might not adequately reflect the changes occurring. Accordingly, the length of phase 1 showed differences between species; thus, in geese it was 8–11 d (Le Maho et al. 1981; Boismenu et al. 1992); in penguins, 2–4 d (Cherel et al. 1988*a*); in quail, 2–3 d (Sartori et al. 1995); and in barn-owls, less than 1 d (Handrich et al. 1993*b*). Nevertheless, the next two phases were similar to the classic model, with a clear increase in daily body-mass loss the last day (phase 3). Totzke et al. (1999) described a fasting experiment with a very similar species (*Larus argentatus* Pontoppidan), but their results showed a body-mass change pattern differing from the classic model. Their birds fasted during a previously fixed period of 6 or 9 d (in two different experiments), and their daily body-mass loss did not rise at the end of the experiment (corresponding to phase 3). The differences among individuals reported here could explain this apparently contradictory result because most of the gulls in the Totzke et al. (1999) study could not have been able to reach phase 3 before refeeding. Totzke et al. (1999) cited Boismenu et al. (1992) as the only study with a similar result, but this work showed a statistically significant rise in daily body-mass loss in the last phase. Moreover, Totzke et al. (1999) used a pool of adults and yearlings, and the effect of this factor was not controlled, although they concluded that adults could cope better with fasting conditions.

With regard to plasma biochemistry, in this study, urea and uric acid levels changed throughout the deterioration period in fasted gulls in a similar way to the classic model (e.g., Cherel and Le Maho 1985; Sartori et al. 1995; see Fig. 1), with a slight decrease at the beginning (phase 1; see ranks 0%–5% in Fig. 5), followed by a slow increase (phase 2), more abrupt in the last two ranks of total body-mass loss (16%–25%) of the deterioration period (phase 3). Changes in triglyceride concentrations in this group were similar to those changes of free fatty acids and $\beta$-hydroxybutirate observed in the classic model (Groscolas 1986; Cherel et al. 1987; Boismenu et al. 1992; Fig. 1) but differed from the predicted pattern for this lipid (see Fig. 1). It increased at first and then declined close to the deterioration limit. Triglycerides are positively correlated with

the increment in body mass of captive garden warblers (*Sylvia borin*; Jenni-Eiermann and Jenni 1994) and with the weight of body fat in turkeys (*Meleagris gallopavo*; Bacon et al. 1989) and mallards (*Anas platyrhynchos*; Dabbert et al. 1997). However, our triglyceride concentration analyses included amounts of free glycerol. Triglycerides are the precursors of glycerol, free fatty acids, and, finally, ketone bodies such as $\beta$-hydroxybutirate (Griminger 1986). Therefore, the observed increment can reflect the fat reserve mobilization. In herring gulls, Totzke et al. (1999) showed an increment in triglycerides (also including free glycerol) and $\beta$-hydroxybutirate levels like in phase 2, but their concentrations did not decrease when the fasting period ended, which would seem to support the idea that phase 3 was not reached in their experiment. In spite of this, Totzke et al. (1999) suggested a minor resistance to starvation in gulls. However, our results suggest that adult yellow-legged gulls show a moderate adaptation to prolonged fasting, with a biochemical pattern very similar to the classic model but with great differences between individuals.

Table 2: Relationship of each biochemical parameter with the group (fasting or restricted) and with the proportion of total body-mass loss (TBML)

| | Deterioration Period | | Recovery Period | |
|---|---|---|---|---|
| Biochemicals | Group | TBML % | Group | TBML % |
| Urea | 1.92 | 149.08*** | 8.86* | 8.76** |
| Uric acid | 3.75 | 57.72*** | .01 | .84 |
| Triglycerides | 4.73* | 27.88*** | 5.51* | 1.48 |
| Total protein | 1.88 | 9.83** | .01 | 66.89*** |
| Creatinine | 5.26* | 47.53*** | .01 | 16.99*** |
| Pi | .03 | 20.37*** | .04 | 2.86 |
| Ca | 2.38 | 14.46*** | .34 | 80.26*** |
| Mg | .05 | 4.43* | .15 | 13.39*** |
| Glucose | .79 | 16.57*** | 1.47 | 3.34 |
| Cholesterol | .79 | 164.02*** | 1.22 | 10.71** |
| Amylase | 4.04* | 6.10* | .42 | .07 |
| AP | .88 | .16 | 0 | .19 |

Note. *F* values from variance analysis (general linear model). Dependent variable: each biochemical parameter. Fixed factor: group. Covariable: TBML %. Random factor: individual ($P < 0.001$ in every biochemical parameter). $P > 0.05$: not significant; $n_{deterioration} = 102$; $n_{recovery} = 88$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

### *Strategy of Subfed Gulls*

The second objective of this study was to compare the changes during periods of moderate food restriction with respect to periods of absolute fasting. The restricted group showed differences with respect to the fasting pattern. In this way the subfed gulls tended to maintain a lower level of daily body-mass loss throughout the deterioration period than did fasting gulls (Fig. 2). Variation of uric acid was different among the treatments in the deterioration period because it started to increase in the restricted group in advance, in a constant way (Fig. 3). High levels of proteins in the diet can increase uric acid levels in birds (Okumura and Tasaki 1969; Lumeij and Bruijne 1985). Hence, a fish diet might have increased the nitrogen wastes from the beginning of the experiment and later,

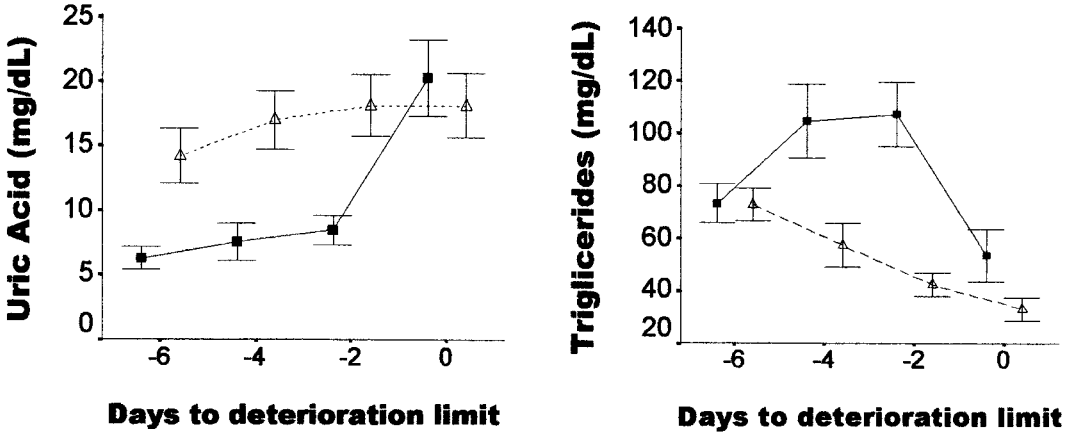


Figure 3. Comparisons between the fasting and restricted groups in urea (*left*) and triglyceride (*right*) plasma concentrations throughout the deterioration period (food restriction). Individual data synchronized backward from the last day of this period. Solid squares and continuous lines represent gulls in the fasting group, and open triangles and dotted lines represent the restricted gulls group. Proposed phases marked over graphics. (Means ± SE; $n = 9$, both groups.)

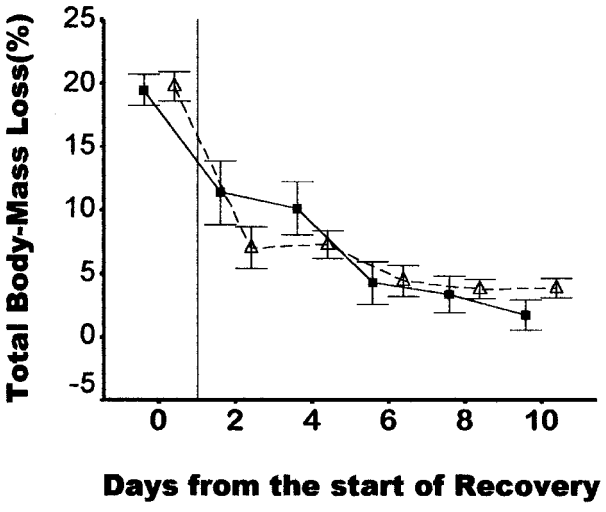


Figure 4. Variation in total body-mass loss (body-weight proportion with respect to the first experimental day) backward from the last day of the deterioration period (divided by the vertical line) and throughout the recovery period. Solid squares and continuous lines represent gulls in the fasting group, and open triangles and dotted lines represent the restricted gulls group. (Means ± SE; fasting group: $n = 5$; restricted group: $n = 8$.)

in addition to the product from structural or muscular protein catabolism. Triglycerides were also different among the treatments in the deterioration period. The restricted group did not show a clear increment from the beginning and decreased regularly throughout the period. Hence, we propose a model of resources allocation in these subfed gulls: the birds might directly use food protein as an energy resource, consuming body fat slowly throughout the deterioration.

Changes in other biochemical parameters might give more information in this sense. Higher levels of total protein, creatinine, inorganic phosphorus, magnesium, and calcium in fasting birds during the last phase of the deterioration period could reflect a higher level of health damages in the restricted group (see Fig. 5). Some studies have shown a regular maintenance of total protein levels during fasting (Jeffrey et al. 1985; García-Rodriguez et al. 1987; Jenni-Eiermann and Jenni 1994). Nevertheless, Boismenu et al. (1992) described a decline in total protein in phase 3 in the greater snow geese (*Chen caerulescens atlantica*), a bird that endures a month without food, and Totzke et al. (1999) showed a sustained lowering of total protein levels in herring gulls throughout 6 d. According to Boismenu et al. (1992) and Totzke et al. (1999), in the fasting group the protein levels should decline. In contrast, the total protein level seems sustained. This might be explained because protein levels in fasting birds were maintained by dehydration (Augustine 1982; Rosskopf et al. 1982). In this way, the increase of plasma creatinine, inorganic phosphorus, and magnesium shown here is related to renal malfunction (Rosskopf et al. 1982; Brugère-Picoux et al. 1987), which supports the proposed higher deterioration in the fasting group in phase 3. Calcium was highly related to protein (Spearman coefficient: $r = 0.90$, $P < 0.001$; $n = 18$; in order to avoid pseudoreplication, mean values for each individual were used). This positive correlation was described in other birds (Lumeij 1990; Lumeij et al. 1993) and was related to the transport of total calcium by plasma proteins. A strong relationship to the metabolism of the muscle might be suggested in this sense.

### *Changes in Other Biochemical Traits throughout the Experiment*

Other substances that were not used to explain the deterioration models could clarify the significance of the exposed results. Glucose was normally described as stable during the fast (e.g., Groscolas and Rodriguez 1981; Jeffrey et al. 1985; Totzke et al. 1999) or showing a soft drop close to the beginning, to maintain the first levels throughout the experiment (Sartori et al. 1995). In contrast, Cherel et al. (1988*b*) and Boismenu et al. (1992) showed a sudden decrease in phase 3. Maintenance of glucose is critical for homeotherms because many tissues and cells depend on blood glucose (e.g., the central nervous system; see Castellini and Rea 1992). Nevertheless, in this work a regular decline in glycemia occurred in both groups (see Fig. 5). The yellow-legged gull might be a species that uses other energy sources in the first stages of the fast, such as ketone bodies, which would decrease glucose requirements. Thereby, the difference in effectiveness of the gluconeogenetic pathway among different species of birds has been previously suggested (Ferrer et al. 1987). Alternatively, these species could not properly adapt to food restriction, as suggested before by the body-mass change and by Totzke et al. (1999).

Cholesterol was strongly related to total body-mass loss over the experiment. A positive relationship between cholesterol and body mass has been described in growing black ducks (*Anas rubripes*) in artificially poor ecological conditions (Rattner et al. 1987). However, in other gull species cholesterol remained stable during fasting, pathological processes, and migration (Jeffrey et al. 1985; Averbeck 1992; Cantos et al. 1994). In herring gulls, Totzke et al. (1999) also described a stable pattern of cholesterol levels, but before fasting, their gulls were fed with commercial chicken feed, which could create lower plasma values with respect to the natural populations and lesser possibilities of decline during fasting. An increment in diet protein produces a rise in the synthesis of cholesterol in the liver and intestines but a reduction in plasma concentration, while a low-protein diet causes a high plasma cholesterol level, reducing its excretion (Leveille and Sauberlich 1961; Yeh and Leveille 1972; Lewandowski et al. 1986). Cholesterol contributes to the intestinal absorption of nutrients because it is part of biliar acids and takes part in the construction of cell membranes, especially

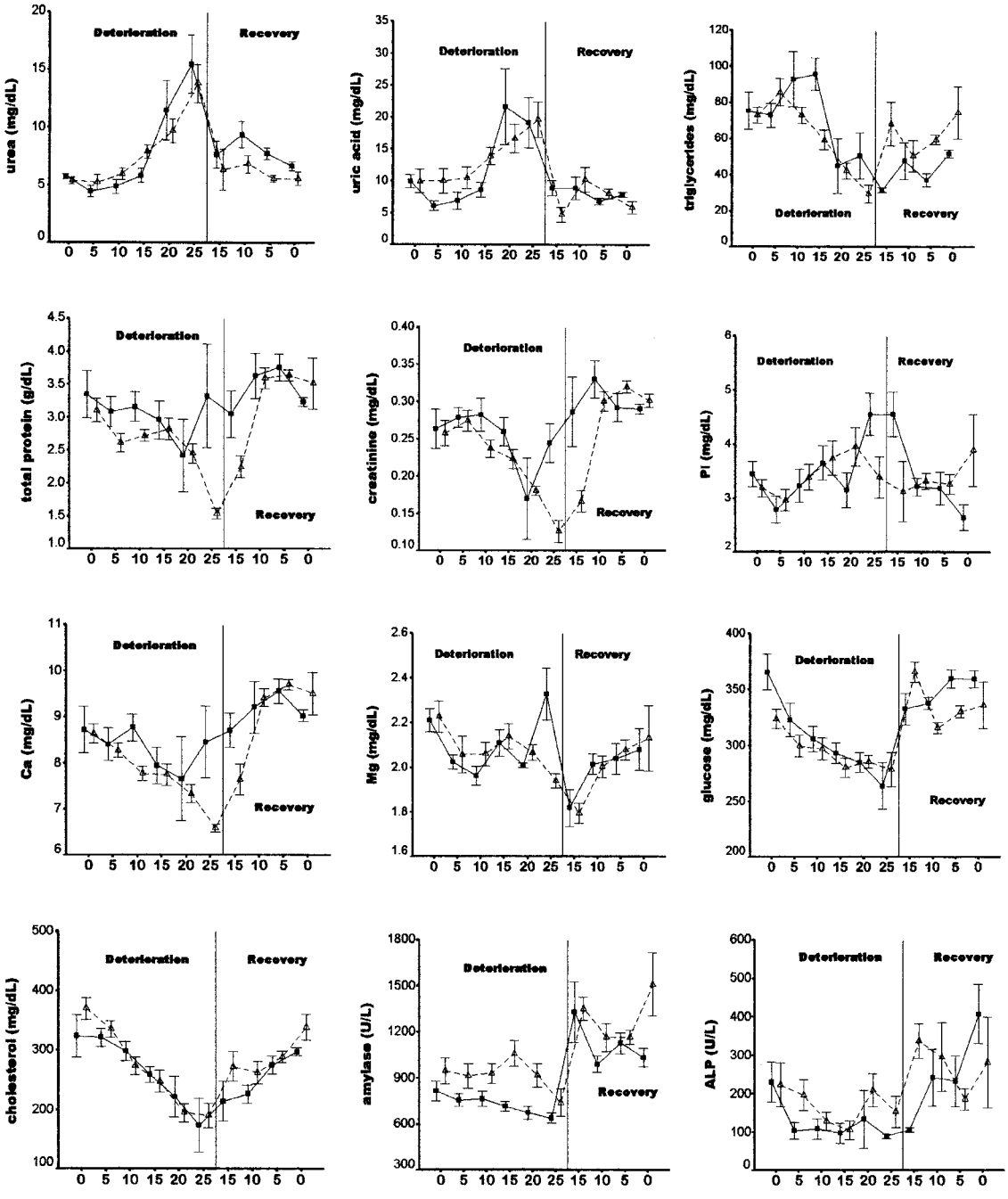


Figure 5. Variation on different plasma biochemical parameters (means ± SE) in relation to total body-mass loss (body-weight proportion with respect to the first experimental day). Proportions are divided in ranks of 5% separately in the deterioration period (food restriction) and the recovery period (food ad lib.). Ranks from 1% to 5% are represented with number 5, and so on. Because these are not the cases, they do not show intermediate ranks between the last rank of the deterioration period and the first of the recovery period. Solid squares represent gulls in the fasting group, and open triangles represent the restricted gulls group. (Deterioration period: $n = 9$, both groups; recovery period: $n = 5$, fast group; $n = 8$, restricted group.)

during growth (Griminger 1986). In this way, the cholesterol decline shown here could suggest a decline in anabolic processes. This synthesis-excretion relationship should explain the slow decline and recovery of cholesterol levels throughout the experiment and could attenuate the differences among the groups.

*Recovery Period*

The third objective of our study was to analyze the recovery process in a bird species. Our yellow-legged gulls quickly recovered most of their total body-mass loss proportion (in only 2 d). After this jump, body weight continued to be more or less stable but below the initial level of the experiment. In contrast, Handrich et al. (1993*a*) found a slower mass recovery in the barn owl *Tyto alba* Scopoli that lasted 8 d, which they named the "refeeding 1" phase. The birds' energy requirements for body-mass recovery decreased throughout the cited phase. In our study, many plasma parameters quickly recovered their initial values after refeeding (see Fig. 5), while other traits showed many changes before the experiment ended (see Fig. 5). This could mean that gulls did not reach their prefasting steady metabolism even though they quickly recovered their initial body mass. Glucose represents the clearest difference among the periods, with a quick recovery and stabilization, which reflected the importance of this carbohydrate in metabolism (Hazelwood 1986). Urea and uric acid also recovered initial levels quickly, in agreement with the findings in refed chickens, which recovered initial values in a matter of hours (Okumura and Tasaki 1969), but in contrast to the very slow recovery tendency shown in buzzards (García-Rodriguez et al. 1987). Nevertheless, this must be considered the sample interval in this study. However, urea and triglyceride levels differed between groups (Table 2): restricted birds regained their initial values at a faster rate (Fig. 5). Again, this result supports the hypothesis that absolute fasting affects gulls' health.

Finally, enzyme levels showed an increase with respect to the deterioration period, probably due to the digestive action of amylase on the food intake (Duke 1986) or the participation of alkaline phosphatase in intestinal absorption (Bell 1971). Amylase is secreted by the pancreas in response to an intestinal stimulus. Production of this enzyme decreases during fasting in chickens and increases when food is restored (Kokue and Hayama 1972). This was quantified directly by means of a catheter. However, as far we know, these changes have never been reported in bird plasma. Moreover, knowledge about this enzyme in birds has not changed much in the past 14 yr (see reviews in Duke 1986 and Denbow 2000). Accordingly, alkaline phosphatase activity falls in the intestine during starvation and is partially restored by refeeding in cockerels (Majumdar and Panda 1989), though this pattern was never reported in birds' plasma before this study.

## Conclusions

Yellow-legged gulls showed a moderate physiological adaptation to extended fasting but the same model of biochemical changes in plasma that other more adapted species showed. Subfed birds seem to use lipids and proteins in a different way than fasted birds, probably suffering a lesser impact on their health. The gulls quickly recovered body mass during the refeeding period, but whereas some plasma substances quickly reached their initial values, others showed many changes before the experiment end, which could reflect a process of metabolic restabilization. Some differences in the results reported here with respect to a recent study on fasting in herring gulls can be explained by methodological interferences.

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## Literature Cited

Augustine P.C. 1982. Effect of feed and water deprivation on organ and blood characteristics of young turkeys. Poult Sci 61:796–799.

Averbeck C. 1992. Haemathology and blood chemistry of healthy and clinically abnormal great black-backed gulls *Larus marinus* and herring gulls *Larus argentatus*. Avian Pathol 21:215–223.

Bacon W.L., K.E. Nestor, and E.C. Naber. 1989. Prediction of carcass composition of turkeys by blood lipids. Poult Sci 68: 1282–1288.

Bell D.J. 1971. Plasma enzymes. Pp. 963–971 in D.J. Bell and B.M. Freeman, eds. Physiology and Biochemistry of the Domestic Fowl. Academic Press, New York.

Boismenu C., G. Gauthier, and J. Larochelle. 1992. Physiology of prolonged fasting in greater snow geese *Chen caerulescens atlantica*. Auk 109:511–521.

Brugère-Picoux J., H. Brugère, I. Basset, N. Sayad, J. Vaast, and J.M. Michaux. 1987. Biochemie clinique en pathologie aviaire: intérêt et limites des dosages enzymatiques chez la poule. Recl Med Vet 163:1091–1099.

Cantos F.J., A.L. Alonso-Gómez, and M.J. Delgado. 1994. Seasonal changes in fat and protein reserves of the black-headed gull, *Larus ridibundus*, in relation to migration. Comp Biochem Physiol 108A:117–122.

Castellini M.A. and L.D. Rea. 1992. The biochemistry of natural fasting at its limits. Experientia 48:575–582.

Cherel Y. and Y. Le Maho. 1985. Five months of fasting in king penguin chicks: body mass loss and fuel metabolism. Am J Physiol 249:R387–R392.

Cherel Y., J-P. Robin, and Y. Le Maho. 1988*a*. Physiology and biochemistry of long-term fasting in birds. Can J Zool 66: 159–166.

Cherel Y., J.-P. Robin, O. Walch, H. Karmann, P. Netchitailo, and Y. Le Maho. 1988*b*. Fasting in king penguin. I. Hormonal and metabolic changes during breeding. Am J Physiol 254: R170–R177.

Cherel Y., J-C. Stahl, and Y. Le Maho. 1987. Ecology and physiology of fasting in king penguin chicks. Auk 104:254–262.

Cramp S. and K.E.L. Simmons. 1983. Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of Western Palearctic. Vol. 3. Waders to Gulls. Oxford University Press, Oxford.

Dabbert C.B., T. E. Martin, and K.C. Powell. 1997. Use of body measurements and serum metabolites to estimate the nutritional status of mallards wintering in the Mississippi Alluvial Valley, U.S.A. J Wildl Dis 33:57–63.

Denbow D.M. 2000. Gastrointestinal anatomy and physiology. Pp. 299–325 in G.C. Whittow, ed. Sturkie's Avian Physiology. Academic Press, London.

Duke G.E. 1986. Alimentary canal: secretion and digestion, special digestive functions, and absorption. Pp. 289–302 in P.D. Sturkie, ed. Avian Physiology. Springer, New York.

Felig P. 1979. Starvation. Pp. 1927–1940 in L.J. De Groot, ed. Endocrinology. Grune & Stratton, New York.

Ferrer M. 1993. Blood chemistry studies in birds: some applications to ecological problems. Trends Comp Biochem Physiol 1:1031–1044.

Ferrer M., T. García-Rodríguez, J.C. Carrillo, and J. Castroviejo. 1987. Hematocrit and blood chemistry values in captive raptors (*Gyps fulvus*, *Buteo buteo*, *Milvus migrans*, *Aquila heliaca*). Comp Biochem Physiol 87A:1123–1127.

García-Rodríguez T., M. Ferrer, J.C. Carrillo, and J. Castroviejo. 1987. Metabolic responses of *Buteo buteo* to long-term fasting and refeeding. Comp Biochem Physiol 87A:381–386.

Griffiths R., M.C. Double, K. Orr, and R.J.G. Dawson. 1998. A DNA test to sex most birds. Mol Ecol 7:1071–1075.

Griminger P. 1986. Lipid metabolism. Pp. 345–358 in P.D. Sturkie, ed. Avian Physiology. Springer, New York.

Groscolas R. 1986. Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. J Comp Physiol 156B:521–527.

Groscolas R. and A. Rodriguez. 1981. Glucose metabolism in fed and fasting emperor penguins. Comp Biochem Physiol 70A:191–198.

Handrich Y., L. Nicolas, and Y. Le Maho. 1993*a*. Winter starvation in captive barn-owls: bioenergetics during refeeding. Auk 110:470–480.

———. 1993*b*. Winter starvation in captive barn-owls: physiological states and reversible limits. Auk 110:458–469.

Hazelwood R.L. 1986. Carbohydrate metabolism. Pp. 303–325 in P.D. Sturkie, ed. Avian Physiology. Springer, New York.

Jeffrey D.A., D.B. Peakall, D.S. Miller, and G.R. Herzberg. 1985. Blood chemistry changes in food-deprived herring gulls. Comp Biochem Physiol 81A:911–913.

Jenni-Eiermann S. and L. Jenni. 1994. Plasma metabolite levels predict individual body mass changes in a small long-distance migrant, the garden warbler. Auk 111:888–899.

Kokue E. and T. Hayama. 1972. Effects of starvation and feeding in the endocrine pancreas of chicken. Poult Sci 51: 1366–1370.

Le Maho Y., H. Vu Van Kha, H. Koubi, G. Dewasmes, J. Girard, P. Ferré, and M. Cagnard. 1981. Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. Am J Physiol 241:E342–E354.

Leveille G.A. and H.E. Sauberlich. 1961. Influence of dietary protein level on serum protein components and cholesterol on growing chick. J Nutr 74:500–504.

Lewandowski A.H., T.W. Campbell, and G.J. Harrison. 1986. Clinical chemistries. Pp. 192–200 in G.J. Harrison and L.R. Harrison, eds. Clinical Avian Medicine and Surgery. Saunders, Philadelphia.

Lumeij J.T. 1990. Relation of plasma calcium to total protein and albumin in African grey *Psittacus erithacus* and Amazon *Amazona spp.* parrots. Avian Pathol 19:661–667.

Lumeij J.T. and J.J. Bruijne. 1985. Blood chemistry reference values in racing pigeons (*Columba livia domestica*). Avian Pathol 14:401–408.

Lumeij J.T., J.D. Remple, and K.E. Riddle. 1993. Relationship of plasma total protein and albumin to total calcium in peregrin falcons *Falco peregrinus*. Avian Pathol 22:183–188.

Majumdar S. and J.M. Panda. 1989. The activities of phosphatases and aminotransferases in the epithelium of the small intestine and caecum of white rock cockerels during starvation. Zentralbl Veterinaermed A 36:348–353.

Munilla I. 1997. Estudio de la población y ecología trófica de la gaviota patiamarilla (*Larus cachinnans*) en Galicia. PhD thesis. Universidade de Santiago de Compostela, Spain.

Norusis M.J. 1993. SPSS Base 6.0 Applications Guide. SPSS, Chicago.

Okumura J. and I. Tasaki. 1969. Effect of fasting, refeeding and dietary protein level on uric acid and ammonia content of blood, liver and kidney in chickens. J Nutr 97:316–320.

Rattner B.A., G.M. Haramis, D.S. Chu, C.M. Bunk, and C.G. Scanes. 1987. Growth and physiological condition of black ducks reared on acidified wetlands. Can J Zool 65:2953–2958.

Rosskopf W.J., R.W. Woerpel, G.A. Rosskopf, and D. Van DeWater. 1982. Hematological and blood chemistry values of commonly kept cockatoos. Calif Vet 36:9–15.

Sartori D.R.S., R.H. Migliorini, J.A.S. Veiga, J.L. Moura, I.C. Kettelhut, and C. Linder. 1995. Metabolic adaptations induced by long term fasting in quails. Comp Biochem Physiol 111A:487–493.

Totzke U., M. Fenske, O. Hüppop, H. Raabe, and N. Schach. 1999. The influence of fasting on blood and plasma composition of herring gulls (*Larus argentatus*). Physiol Biochem Zool 72:426–437.

Yeh S.-J.C. and G.A. Leveille. 1972. Cholesterol and fatty acid synthesis in chicks fed different levels of protein. J Nutr 102: 349–358.