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Tracking forced moult by computer tomography and serum biochemical parameters in laying hens

Verfolgung der Zwangsmauser bei Legehennen mit Hilfe von Computertomographie und biochemischen Blutparametern

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Introduction

Forced moulting, nowadays, is a less often used technological process in egg production in the European area, than it was at the eighties (Ballay et al., 1984). In contrast, in the Northern American egg production it still plays an important role. The avoidance of forced moulting is based on the relatively high stress, arising mainly from total feed withdrawal, which the animals are exposed to (Keshavarz and Quimby, 2002). In the past few years because of animal welfare aspects moulting techniques applied are questioned. Consequently, interest in alternative methods to induce moulting has increased (Ruszler, 1998). It was described earlier (Rolon et al., 1993) that total feed deprivation is not needed, when low-energy diet is fed (so-called non feed removal methods) (Biggs et al., 2003).

However, as forced moult is associated with intensive body weight loss, this process is highly interesting both from biochemical aspects (Gildersleeve et al., 1983), and from the viewpoint of body composition.

The marked changes of different organs were reported in detail by Brake and Thaxton (1979b) during feed withdrawal and also in the following, refeeding period. In addition, Gildersleeve et al. (1983) determined alterations of numerous serum metabolites and enzymes. From the above-mentioned studies it can clearly be seen that during partial or total feed and/or water restriction, the determined biochemical markers react sensitively.

The current study was aimed to follow a 12-day-long period of forced moult of Hy-Line® hens by total feed withdrawal. According to Buhr and Cunningham (1994), the postmoult performance does not depend on the severity of body weight loss (15, 20 or 25%) in the moulting period. The main aspect that feed withdrawal accompanied by a short-daylight regime and a long recovery period generally leads to the best postmoult production (Bar et al., 2001) has been taken into account. Therefore, the effects of the moulting procedure were followed by repeated blood samplings and also by CT scanning, for a precise description of the process.

The *in vivo* CT imaging procedure applied in the current study was also used by Bentsen and Sehested (1989), Svihus and Katle (1993), and Romvári et al. (1994) for the determination of the abdominal fat amount and the breast muscle weight of broiler chickens. Body composition analysis of growing broiler chickens were performed by means of dual X-ray absorptiometry and by HRCT imaging (Mitchell et al., 1997; Andrássy et al., 2003b). The comparative investigation of the breast muscles of different broiler genotypes, complemented with 3D reconstruction was performed by means of HRCT imaging by Romvári et al. (2000).

The joint approach was aimed to follow-up the metabolism during moulting (blood parameters) complemented with a description of the fat breakdown in a precisely localized manner (CT imaging).

Materials and methods

Experimental animals

In total, 27 Hy-Line Brown laying hens kept in cages were randomly selected for the study at the age of 608 days with a body weight of 2.04±0.07 kg. Hens were placed on deep litter, and from the 1st day feed was totally withdrawn, while water was offered ad libitum. The light intensity was set to 8 lux in an 8-hour period with 16 hours of darkness while the temperature was 14±1.2 °C. Before the moulting experiment, the light program used week was: 16 h/day, 3W/m^2, from 05 to 21 h daily. Body weight measurements and blood samplings were performed every three days (0, 3, 6, 9 and 12 days of moulting). After the last blood sampling (day 12) the animals were refed, ad lib. (ME = 11.45 MJ/kg, CP = 16.75%, CF = 2.95%, crude fiber = 3.55%) for a regeneration period of 3 weeks.

CT scanning

The CT scanning was carried out at the Institute of Diagnostic Imaging and Radiation Therapy, on a Siemens Somatom S40 apparatus. The examination was performed in a repeated manner at the beginning of the moulting period, the 6th and 12th days of it and finally three weeks after the course. Birds were scanned in a prone position, with stretched legs without anesthesia. 32 cross-sectional consecutive scans of 10 mm slice thickness with a zoom factor of 2.5 covered the total body.

The image processing was double aimed. The determination of the total body composition was performed on the basis of the Hounsfield (HU) scale. According to this, the muscle and the fat density pixels (-200 – +200 HU) were computed from all images. Afterwards, the proportional changes of the different tissue types (fat, inner organs and

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muscle were defined as: HU -150 – -61, HU -60 – $^{+}$39 and HU $^{+}$40 – $^{+}$139) were determined, by taking the slice thickness and the zoom factor into account.

Forty so-called Hounsfield variables (HUv) were calculated from the frequency values of the pixels with the summing of the consecutive 10-10 HU values (fat, inner organs and muscle were defined as: HUv 6-14, HUv 15 - 24 and HU 25 -34). Due to practical reasons (i.e. better surface rendering) all the forty variables were rendered adding that, under 6 and over 34 HUv no peak is present. The 3D surface smoothing was developed by the "NURBS" method. The interpolation was done using a conventional B-spline algorithm (HARTLY and JUDD, 1980).

Blood sampling

Form the *v. brachialis* 1.5 ml samples were collected and were allowed to clot on room temperature. Serum was obtained from centrifugation at 5500 r/min for 10 min, and 0.5 ml serum aliquots were stored frozen. Serum measurements were performed at the end of trial, on a Konelab® 20i automated apparatus, within the same analytical run. Serum total protein (TP), albumin (ALB), uric acid, triacylglycerol (TAG), total cholesterol (CHOL), HDL-cholesterol, creatinine (CREA), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ -GT), aspartate aminotransferase (AST) and inorganic phosphorus (Pi) were determined every 3 days. Serum lactate dehydrogenase (LDH), alanine aminotransferase (ALT), Ca, K, Cl, Na and Fe were determined also. In all cases Konelab® reagents were used.

Statistical analysis

In the evaluation of serum parameters multivariate analysis of variance was applied. Time was set as fix factor while body weight as covariate. The consecutive measurements were compared with the least significant difference (LSD) „post hoc" test. SPSS 10 for WINDOWS (1999) was used.

Results and discussion

Body weight

Body weight strongly decreased by time (days 0; 3; 6; 9 and 12: 2.04 ± 0.07; 1.95 ± 0.09; 1.82 ± 0.11; 1.66 ± 0.09; 1.62 ± 0.09 kg, respectively). The achieved 21.6% weight loss was found to be succesfull to introduce the moulting process and therefore the trial was terminated according to BUHR and CUNNINGHAM (1994), without any looses in the hen population.

CT-determined body composition

The total body composition has been plotted on the basis of the calculated HUvs, covering the fat and muscle interval. The serial number of the serial cross sectional CT scans is marked on the on X-axis, the HU variables on the Y-axis (numbering from 1 to 40, after reducing by 10 from -200 to $^{+}$200) and the frequency of density values were indicated on the Z-axis. For the proper comparison of the scale of all three axes is always identical.

In Figure 1 two dominant peaks are visible, both within the muscle density interval (HUv 24 - 34). As marked in the picture, one represents the pectoral muscle area with the wings, while the next the thigh musculature. In the fat density interval (HUv 6 - 14), two marked depots were detected: one at the neck and a relatively large depot within the abdomen (as marked in the picture). In the area between muscle and fat (around the zero density value, HUv 0) so-called "mixed voxels" are present, consisting of both fat and muscle density tissue volumes. (These mixed volume units (voxels) arise due to the fact that the physical resolution of CT is 1 x 1 mm x 10 mm slice thickness (10 mm^3) while fat-muscle borderlines are below this size, resulting in mean-density valued voxels.) This phenomenom was described by CHOI et al. (1991) in detail.

Within only 6 days marked decreases could be observed in the fat depots, while an additive peak (protrusion) was detected. This latter was clearly attributable to the mixed voxel volume, increasing along the moulting process. The location of the marked peak (scans 15-21) indicates a process in the abdomen, meaning an average density increase, in close connection with the decrease of the fat volume (Figure 2).

As shown in Figure 3 fat depots are strongly reduced, the shape of the 3D model is only one-peaked, being characteristic for a lean body. At this stage parallel with the approximately 20% body weight loss intensive moulting started, therefore it was decided to finish the treatment.

The difference of the initial and final stages in body composition was demonstrated according the method described by ANDRÁSSY et al. (2003a), on growing heavy type turkeys. This method is based on the biometrical processing of the difference of the frequency distribution developed in the two time points.

The cavities in the 3D model (Figure 4) indicate the strong decreases experienced in the fat depots, as well as

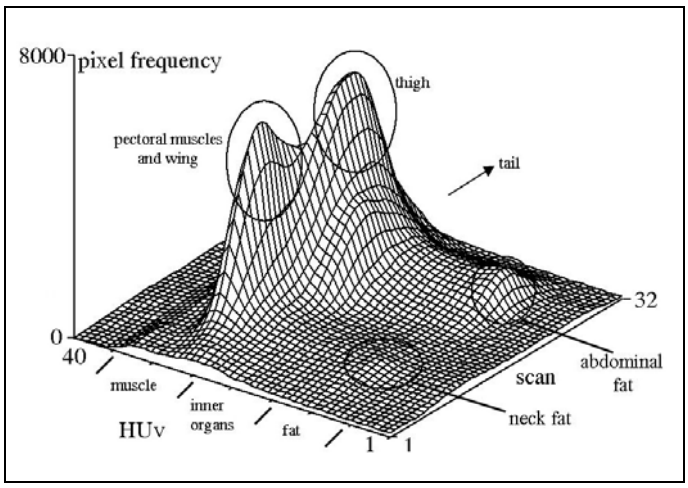

Figure 1. 3D surface of the hens' total body composition before moulting
3D-Darstellung der Gesamtkörperzusammensetzung der Hennen vor dem Beginn der Mauser

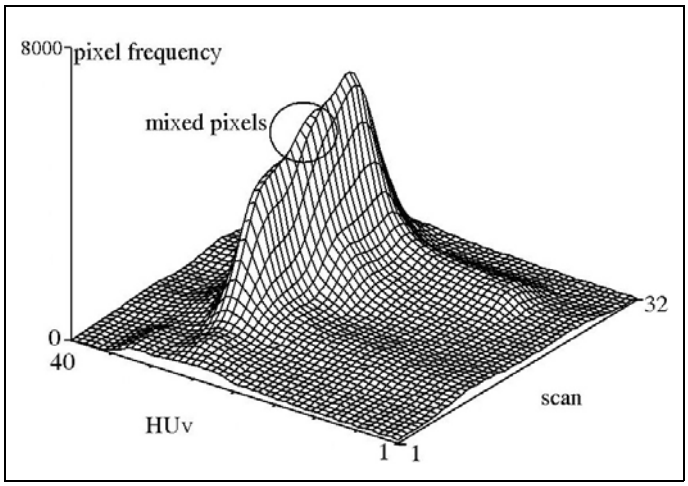

Figure 2. 3D surface of the hens' total body composition on the 6th day of moulting
3D-Darstellung der Gesamtkörperzusammensetzung der Hennen 6 Tage nach dem Beginn der Mauser

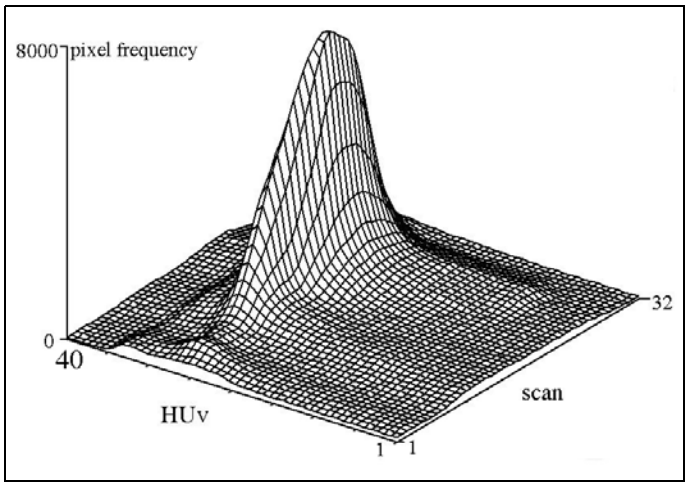

Figure 3. 3D surface of the hens' total body composition on the 12th day of moulting
3D-Darstellung der Gesamtkörperzusammensetzung der Hennen 12 Tage nach dem Beginn der Mauser

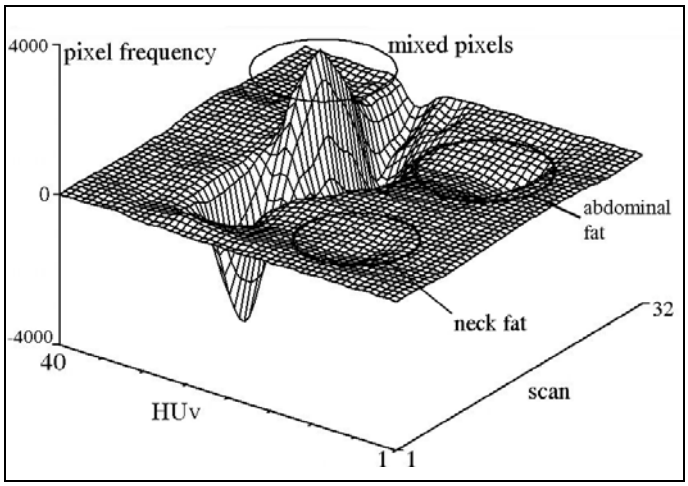

Figure 4. Changes in body composition during the whole moulting period
Veränderungen der Körperzusammensetzung über die gesamte Mauserperiode

in the abdominal fat, supporting the well known high and positive correlation between abdominal fat and other lipid depots (CHAMBERS, 1990). According to the findings described earlier, the new peak between scans 17 and 24 indicates the fat loss of the abdominal organs, leading to an increase of the average density in this area. Moreover, further volumetric reduction in the characteristic muscle density interval was detected, due to the density change caused by the fat content decrease of muscle tissue.

As shown in Figure 5 a three-week regeneration period by ad libitum feeding, after the cessation of feed withdrawal, seemed to be enough to reach the initial body composition condition of the hens. The double peak, characteristic for the muscles and that of fat deposits are clearly to be identified.

The above-demonstrated differences were depicted in Figure 6. showing the changes of the tissue composition in percentage.

Data obtained at the initial stage were taken as 100%. Compared to this, at the 6th and 12th days of moulting the fat depots, the abdominal organs and the muscle tissue were reduced to 52.5, 59.7, 85%, and 29.5, 43.5 and 77.9%, respectively. Following the regeneration period of 3 weeks respective values were 89.6, 100.1 and 86.1%. The

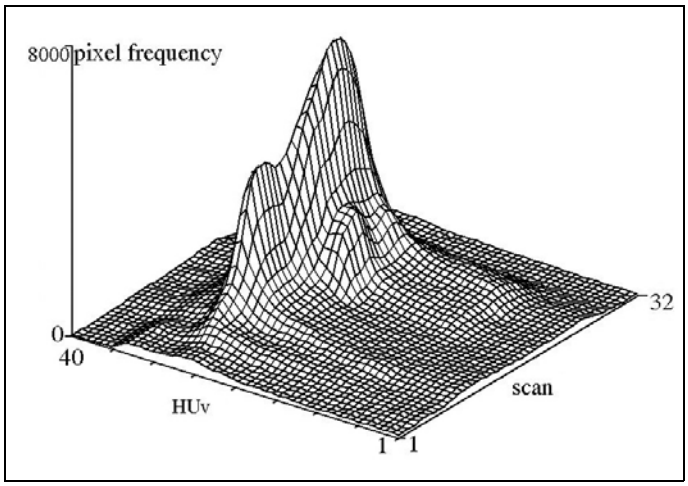

Figure 5. 3D surface of hen total body composition after three weeks of regeneration
3D-Darstellung der Gesamtkörperzusammensetzung der Hennen nach 3 Wochen Regeneration

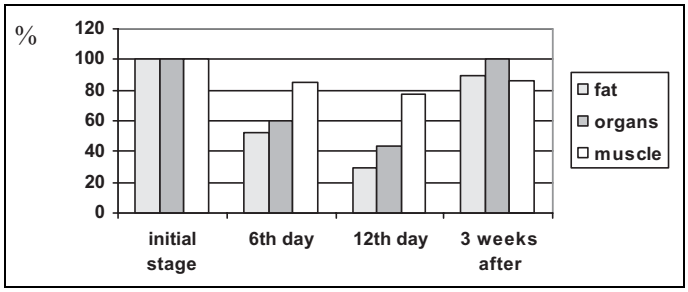

Figure 6. Relative changes of the different tissue categories in the total body of hens during moulting and after 3 weeks recovery
Relative Veränderungen der verschiedenen Gewebe im Körper der Hennen während der Mauser und nach 3 Wochen Regeneration

largest change was found for the fat depots, while the abdominal fat content was the one showing total reloading within the 3 weeks of regeneration.

In a similar manner, comparative investigations of the body composition were carried out on large bodied BUT and bronze type turkeys and also on broiler chickens (Andrássy et al., 2003a, b) where the building of muscle and fat tissue were characterized.

Serum metabolites and enzyme activities

In serum metabolites, HDL cholesterol showed a significant increase, while triacylglycerol a drastic decrease (Table 1). Similarly, total cholesterol concentration increased measurably, which is a known process during prolonged caloric restriction (Schettler, 1983) in humans, as well as in laying hens during moulting (Gildersleeve et al., 1983). Time (elapsed with moulting) had a significant effect on the serum TAG concentration, equivocally indicating the chronic onset of feed withdrawal. A slight decrease was measured in serum chloride (Cl) concentration, though neither weight nor time effect could be detected. Similarly, in the total protein concentration a decreasing tendency was found (without significance). This generally refers to the decrease of the globulins, which is supported by the unchanged albumin concentration and also by the results of Levin et al. (1993), especially in female animals. According to Brake and Thaxton (1979a), the reducion of the ovarian estrogen production leads to a generally lower total protein level. Though hormone concentrations were not measured in this study, the serious decrease of the volume of the inner organs (Figure 6) clearly suggests also the follicular atresia.

Creatinine (CREA) concentration tended to decrease slightly by time. In the background of this finding two factors may stand. One is the reduced activity of the hens due to the increased daily dark period (16 h) and, on the other hand, the strong decrease of the total skeletal muscle volume (Figure 6) in the body. In serum sodium (Na), inorganic phosphate (Pi), uric acid, albumin and total protein no significant alterations were found, sodium mainly referring to the *ad libitum* water supply of the birds.

Sexual quiescence was introduced by the cessation of egg production (within 3 days). The consequent decrease in serum calcium concentration was, at least partly, attributed to the interruption of the egg shell production. Interestingly, in the serum total iron concentration a drastic drop was found, which has, as far as the authors are aware, not been described before.

In enzyme activities (Table 2) alkaline phosphatase (ALP) showed a strongly increasing tendency, though during the 2nd half of the study this turned to a lowering phase; time effect was significant in the model. Furthermore, the lactate dehydrogenase (LDH) activity was significantly increased both by time and weight. In general, the increasing LDH activity reflects the strong catabolic reactions that were also described for the fat depots (Figure 4). These results are both supported by the findings of Gildersleeve et al. (1983). In contrast, neither AST nor γ-GT showed marked alterations.

Conclusions

The same hens could be measured repeatedly during the moulting period, with an average time consumption of 6-8 minutes for the whole-body CT scanning of one hen. The 3D histograms developed clearly show the tissue compositions of the bodies, with separate peaks corresponding to the muscle and fat region. Based on the 3D surfaces, the decrease in the volume of the different tissues and also the regeneration of it can be analyzed during moulting.

Based on the total body composition data it was found that during a 21.6% total body weight loss (in only 12 days) fat volume decrease is predominant (70.5%), which is in three weeks nearly reversible. In contrast, though the

Table 1. Changes of the serum metabolite concentrations in laying hens during moulting
Veränderung der Stoffwechselparameter im Blutserum der Legehennen während der Mauser

	Days of moulting					effects of		
	0	3	6	9	12	bodyweight	time elapsed	bw x te
Albumin [g/L]	20.25 ± 1.98	20.25 ± 1.83	20.00 ± 0.71	19.00 ± 0.71	19.25 ± 1.67	ns	ns	0,059
Total protein [g/L]	59.62 ± 16.85	50.14 ± 6.89	55.43 ± 7.72	47.00 ± 0.00	50.43 ± 5.35	ns	ns	ns
Creatinine [µmol/L]	34.5 ± 8.38^{a}	35.71 ± 4.61^{a}	37.14 ± 4.38^{a}	31.80 ± 4.87^{ab}	28.37 ± 3.38^{b}	0,049	ns	ns
Uric acid [µmol/L]	199.6 ± 55.1	258.4 ± 77.8	202.4 ± 21.6	233.6 ± 60.7	248.9 ± 88.2	ns	ns	ns
Total cholesterol [mmol/L]	1.94 ± 0.40	3.10 ± 1.14	3.79 ± 1.60	3.57 ± 0.12	4.29 ± 0.57	ns	ns	ns
HDL cholesterol [mmol/L]	0.23 ± 0.03^{a}	0.53 ± 0.14^{a}	1.85 ± 0.24^{b}	1.99 ± 0.30^{bc}	2.38 ± 0.31^{c}	0,028	0,083	0,002
Triacylglycerol [mmol/L]	3.57 ± 2.19^{b}	4.48 ± 1.91^{b}	0.92 ± 0.41^{a}	0.47 ± 0.19^{a}	0.27 ± 0.05^{a}	ns	0,011	ns
Inorganic phosphorus [mmol/L]	1.38 ± 0.61	1.24 ± 0.00	1.56 ± 0.50	1.20 ± 0.17	1.13 ± 0.15	ns	ns	ns
Na [mmol/L]	169.2 ± 4.5	167.0 ± 4.0	171.2 ± 4.1	170.7 ± 3.2	165.5 ± 3.1	ns	ns	ns
K [mmol/L]	4.96 ± 0.58^{b}	3.70 ± 0.50^{ab}	3.82 ± 0.15^{a}	4.04 ± 0.21^{ab}	4.79 ± 0.32^{b}	ns	0,005	0,015
Ca [mmol/L]	4.93 ± 1.53^{a}	- ± -	3.17 ± 0.14^{b}	2.80 ± 0.19^{b}	2.71 ± 1.01^{b}	ns	ns	ns
Cl [mmol/L]	133.2 ± 4.05^{ab}	126.2 ± 0.00^{ab}	129.8 ± 4.49^{b}	129.8 ± 4.00^{b}	124.9 ± 3.51^{a}	ns	0,054	0,000
Fe [µmol/L]	66.23 ± 24.41^{a}	- ± -	15.5 ± 2.36^{b}	13.13 ± 7.42^{b}	22.82 ± 6.53^{c}	ns	ns	ns

Significance of differences: a, b: $P < 0.05$

Table 2. Serum enzyme activities in laying hens during moulting (mean±SD)
Aktivitäten der Blutserumenzyme der Legehennen während der Mauser (Mittelwert ± SD)

	Days of moulting					effects of		
	0	3	6	9	12	bodyweight	time elapsed	bw x te
ALP [IU/L]	787.1 ± 629.3^{a}	2160 ± 812.3^{bc}	2304.3 ± 801.0^{c}	1444.6 ± 556.1^{ab}	1263.1 ± 480.1^{ab}	ns	0,040	ns
ALT [IU/L]	3.75 ± 0.96	6.20 ± 4.82	1.29 ± 1.25	2.00 ± 2.65	1.00 ± 0.00	ns	ns	ns
AST [IU/L]	205.7 ± 70.1^{ab}	253.3 ± 59.9^{ab}	116.7 ± 44.3^{a}	274.2 ± 19.7^{b}	228.7 ± 110.4^{b}	ns	0,058	ns
γ-GT [IU/L]	30.60 ± 25.11^{a}	38.25 ± 12.76^{b}	37.83 ± 4.92^{b}	34.20 ± 6.53^{b}	38.50 ± 7.93^{b}	0,087	0,083	ns
LDH [IU/L]	1129.7 ± 836.8^{a}	- ± -	1816.2 ± 469.5^{bc}	1049.0 ± 8.5^{ab}	2518.9 ± 929.9^{c}	0,041	0,010	0,096

Significance of differences: a, b: $P < 0.05$

volume of the inner organs decreased less markedly (56,5% loss), their regeneration was complete in the refeeding period. The lowest volumetric decline was found for the muscle tissue, but regeneration was the least intensive, reaching only 86% of the initial value.

The in vivo methodology applied in our experiment based on CT could be used in comparative experiments aimed at evaluation of different moulting methods.

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Summary

For a complex investigation of forced moulting 27 Hy-Line Brown laying hens were exposed to total feed withdrawal with ad libitum water supply, for a trial period of 12 days. Computer tomography for the determination of the total body composition and its changes was performed on days 0, 6, 12 and after 3 wks of refeeding. Blood samples were collected every 3 days for the monitoring of the metabolic status of the hens. Compared to the initial values at the 6th and 12th days of moulting the volume of fat depots, abdominal organs and muscle tissue were reduced to 52.5, 59.7, 85.0, %, and 29.5, 43.5 and 77.9%, respectively. Following the regeneration period of 3 weeks respective values were 89.6, 100.1 and 86.1%, indicating a nearly fully reversible process. Body composition data were exactly quantified using a 3D smoothing method. Blood metabolic parameters indicated changes referring to total feed deprivation (triacylglycerol, total and HDL cholesterol) and low activity (creatinine) as well as the consequent cessation of egg yolk and shell production (total protein, Ca). The enzymatic adaptation was much less pronounced. It was stated that the in vivo CT scanning may be an effective tool for the comparison of alternative moulting methods.

Key words

Forced moulting, layers, computer tomography, blood serum, metabolite

Zusammenfassung

Verfolgung der Zwangsmauser bei Legehennen mit Hilfe von Computertomographie und biochemischen Blutparametern

Zur komplexen Untersuchung der Zwangsmauser wurde bei 27 Hi-Line Brown Hennen über eine Periode von 12 Tagen das Futter vollständig entzogen, während Trinkwasser weiterhin *ad libitum* zur Verfügung stand. Die Ganzkörperzusammensetzung sowie deren Veränderungen wurden nach 0, 6 und 12 Tagen sowie nach 3 Wochen Regeneration mit Hilfe der Computertomographie erfasst. In regelmäßigen Abständen wurden Blutproben (jeden 3. Tag) gezogen, um die Stoffwechsellage der Hennen zu verfolgen.

Im Vergleich zum Versuchsbeginn (Tag 0) ging das Volumen der Fettdepots, der inneren Organe und des Muskelgwebes bis zum 6. Tag auf 52,5, 59,7 und 85,0% sowie bis zum 12. Tag auf 29,5, 43,5 und 77,9% der Ausgangswerte zurück. Nach der Regenerationsphase von 3 Wochen wurden wieder 89,6, 100,1 und 86,1% der Ausgangswerte erreicht. Die Ergebnisse unterstreichen die Reversibilität des Mauserprozesses. Die Daten der Körperzusammensetzung wurden mit Hilfe einer 3D-Glättungsfunktion (smoothing) quantifiziert. Die biochemischen Stoffwechselparameter im Blut spiegelten den Einfluß des Futterenzugs (Triglyceride, HDL, Cholesterin), die verminderte Aktivität der Hennen (Kreatinin) und die Einstellung der Dotter-und Schalenbildung (Proteine, Ca) wider. Die enzymatische Anpassung war weniger stark augeprägt. Es wurde der Schluss gezogen, dass die Computertomographie eine effektive Methode zum Vergleich verschiedener Mauserprogramme sein kann.

Stichworte

Zwangsmauser, Legehenne, Computertomographie, Blutserum, Stoffwechselparameter

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