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BEHAVIOURAL, ENDOCRINE AND METABOLIC EFFECTS OF FOOD RESTRICTION IN BROILER BREEDER HENS

Ľ. KUBÍKOVÁ, P. VÝBOH, Ľ. KOŠŤÁL

Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences
Ivanka pri Dunaji, Slovak Republic

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Abstract

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The effects of food restriction on behaviour, endocrine and metabolic parameters were studied in immature (13 weeks old) female breeding birds of the meat-type chickens (broiler breeders). *Ad libitum* fed hens were compared with those subjected to one qualitative (diet diluted with 30 % hardwood sawdust) and two quantitative (the daily ration recommended by the breeding company and twice that amount) food restriction treatments. Behaviour of quantitatively restricted hens provided with one daily meal showed marked diurnal variation, while that of hens with free access to food (*ad libitum* fed and qualitatively restricted) was more evenly distributed throughout the day. There were elevated plasma corticosterone concentrations in hens subjected to intensive quantitative restriction. Although an increase in corticosterone concentrations of hens subjected to qualitative food restriction was lower, it was significant in comparison with *ad libitum* control, indicating that even mild qualitative food restriction is stressful. Compared to *ad libitum* fed hens, there were decreased plasma T_3 concentrations in all food restriction treatments, while plasma T_4 concentrations increased in intensively quantitatively restricted hens. Food restriction did not affect plasma glucose and total proteins, whereas triacylglycerol levels were decreased and cholesterol increased as a consequence of restriction. Creatinine level was increased in hens with less intensive quantitative restriction and plasma concentration of uric acid was decreased in qualitatively restricted hens in comparison with all other treatments. These results suggest that although behaviour of qualitatively restricted-fed hens resemble more to that of *ad libitum*-fed hens, their physiological status reminds more quantitatively restricted ones.

Domestic chicken, welfare, corticosterone, thyroid hormones

The major welfare problems in meat type chickens (broilers) are those, which can be regarded as side effects of the intense selection for growth and food conversion. These include leg disorders, contact dermatitis, ascites and sudden death syndrome in growing birds. It is apparent that the fast growth rate of current broiler strains is not accompanied by a satisfactory level of welfare including health (Report of the Scientific Committee on Animal Health and Animal Welfare of the EU Commission 2000).

High body weight of *ad libitum* fed (like the progeny) breeding birds (broiler breeders) is associated with excessive fat deposition, lameness, high mortality associated with skeletal and heart disease (Katanbaf et al. 1989; Savory et al. 1993; Hocking 1999), impaired immune function (Han and Smyth 1972; O'Sullivan et al. 1991; Hocking et al. 1996), and reduced fertility in both sexes (Nestor et al. 1980; Hocking et al. 1987; Hocking and Duff 1989; Robinson et al. 1993). In order to limit the body weight of breeding birds at sexual maturity (about 24 weeks of age), to reduce related health problems, and to increase fertility, the chronic food restriction is applied routinely in commercial conditions. The main welfare problems of broiler breeders are related to the severe food restriction.

Address for correspondence:
Ing. Pavel Výboh, CSc.
Department of Endocrinology and Ethology
Institute of Animal Biochemistry and Genetics SASci
Moyzesova 61, 900 28 Ivanka pri Dunaji, Slovakia

Phone: ++421 2 4594 3232
Fax: ++421 2 4594 3932
E-mail: ubgzvybo@nic.savba.sk
http://www.vfu.cz/acta-vet/actavet.htm

Hens fed on the restricted rations recommended by the breeding companies provided once a day are much more active than *ad libitum* fed control hens and show increased pacing before feeding and increased drinking and pecking at non-food objects afterwards. The expression of these activities varies between individuals, it is often stereotyped in form and correlated positively with the level of food restriction imposed (Košťál et al. 1992; Savory and Maros 1993). Based on pharmacological manipulation of different neurochemical receptor systems it was found that expression of the post-feeding oral stereotypies of restricted-fed broiler breeders was more sensitive to treatment with dopamine receptor agonists and antagonists than to manipulation of other systems (Košťál and Savory 1994; Košťál and Savory 1996; Savory and Košťál 1994). Hypothesis on dopaminergic nature of oral stereotyped behaviour is further supported by increased dopamine levels in basal telencephalon (Košťál et al. 1999) and up-regulation of D_1 and down-regulation of D_2 receptors in several forebrain regions (Košťál et al. 2001) found in restricted-fed broiler breeder hens compared to *ad libitum* fed ones.

Chronic food restriction in broiler breeders causes various hormonal changes, including an increase in plasma concentrations of growth hormone (GH) and thyroxine (T_4) and decrease in plasma concentrations of insulin-like growth factor I (IGF-I) and triiodothyronine (T_3) (Bruggeman et al. 1997; Van der Geyten et al. 1999; Buyse et al. 2000). Changes in GH and IGF-I (Hocking et al. 1994) as well as in thyroid hormones secretion (Bruggeman et al. 1997) have been suggested as possible mechanisms causing differences in egg production between *ad libitum* and food restricted broiler breeders.

Corticosterone is the principal glucocorticoid released by the avian adrenal gland (Whittow 2000) and elevated plasma corticosterone is an accepted indicator of stress condition in birds (Siegel 1995). Chronic food restriction in broiler breeders has been shown to increase corticosterone levels (Savory et al. 1996; Savory and Mann 1997; Hocking et al. 1998). However, care must be taken in interpretation of results, since a rise in concentration is a normal response to fasting and is thus an indicator of physiological state which may or may not be indicative of stress.

Qualitative restriction of nutrient intake by appropriate dietary dilution or appetite suppression, with free access to food, has been suggested as a less stressful alternative to quantitative restriction for limiting growth rate in parent stock of broilers (Savory et al. 1993, 1996). However, suppression of abnormal oral behaviours did not correspond with reduction in blood indices of stress, including corticosterone levels (Savory et al. 1996).

The aim of this study was to examine the effects of qualitative and quantitative food restriction on behavioural, endocrine and metabolic parameters of growing broiler breeder hens and to contribute to the assessment of their welfare status.

Materials and Methods

Twenty four broiler breeder hens Ross 208 (Xaveross, Prague, Czech Republic) were used in the experiment. For the first 2 weeks of life they were kept in cages 60 × 40 × 35 cm (w × d × h) (12 individuals per cage) with food and water provided *ad libitum*. At the age of 2 weeks hens were randomly divided into 4 feeding treatments (6 indivuduals per treatment, 3 individuals per cage). Starting from the beginning of week 7 hens were moved into individual cages measuring 40 × 44 × 41 cm. Cages were arranged in two batteries, each consisting of 7 cages in 3 tiers. Four hens (one per each treatment) were placed into each tier. Empty cages between cages occupied by hens prevented them from reaching feeders of the neighbouring hens. Moreover, cardboard placed inside the empty cages prevented them from seeing each other. Hens belonging to distinct feeding treatments were arranged in batteries in a balanced way. The photoperiod was 23 h light and 1 h dark (23L : 1D) during the first 2 days and decreased progressively to 12L : 12D (06.00 – 18.00 h) at day 6. The temperature was gradually reduced from 30 °C on the first day to 21 °C from 24 days of age onwards.

At 7 weeks of age a starter diet (200 g crude protein per kg, 11.5 MJ kg^{-1} metabolisable energy) was changed to a grower diet (170 g crude protein per kg, 12.0 MJ kg^{-1} metabolisable energy). Both diets were in mash form.

Hens belonging to the first feeding treatment were provided with food *ad libitum* (AL). Hens in the second feeding treatment, qualitative restriction (QR), had free access to food diluted with 30% of hardwood sawdust. Hens belonging to the last two feeding treatments were subjected to quantitative food restriction and received their weighed daily ration once a day at 9.00 h. Hens form the third feeding treatment received twice the amount of daily food ration recommended by the Ross 208 Parent Stock Management Manual (2R) and hens from the fourth treatment received the recommended restricted ration (R). Drinking water was freely available to all treatments. The food consumption (up to 7 weeks of age mean per cage and then individual) was measured daily at 9.00 h, together with refilling of food dispensers. Body weight was recorded once a week.

At the age of 11, 12 and 13 weeks hens were observed once each week during the three 30 min periods commencing 09.30, 13.30 and 17.30 (to take account of diurnal variation in behaviour). Observations were made by two observers. Each of them observed one battery, i.e. 12 hens. Before the start of observation the observers sat quietly for 5 min in front of the cages, to get the hens accustomed to their presence. To be able to see hens in the highest tier, the observers sat on a small ladder. Each hen's behaviour was recorded every minute from a single 'on the dot' (Slater 1978) observation. Following activities were recorded: pecking at feeder (full or empty), drinker directed activity, object pecking, sitting (only or with panting), standing (only or with panting), pacing, preening while sitting and preening while standing. The proportions of time spent in the various activities were calculated from the recordings.

At the end of the experiment at 13 weeks of age, blood samples from the brachial vein were collected using a heparinised syringe. The blood was transferred into chilled tubes and then centrifuged. The plasma was removed and stored at -20 °C until assayed for hormone and metabolic parameters. Corticosterone concentration was determined by radioimmunoassay using the method of Ježová et al. (1994). Thyroid hormones were determined by direct radioimmunoassay without extraction. T_3 was assayed using the method of Földes et al. (1978) and T_4 using commercially available kit (Immunotech, Prague, Czech Republic). Metabolic parameters in plasma (total protein, uric acid, creatinine, glucose, triacylglycerols, cholesterol) were determined with the commercially available kits (Roche, Switzerland) using the Hitachi 911 analyser (Roche, Switzerland).

The effects of age and feeding treatment on body weight were estimated by two-way ANOVA, the effects of testing week, time of day and feeding treatment on the proportion of time spent by distinct behaviours were estimated by three-way ANOVA and the effect of feeding treatment on hormone concentrations and metabolic parameters were estimated by one-way ANOVA. Differences between means were determined by the least significant difference (LSD) procedure.

Results

Body weight of R hens corresponded to the values described in the Ross 208 Parent Stock Management Manual. Body weight of QR hens was between that of 2R and R hens during the most of the studied period, however, at 12 weeks of age it was comparable to that of 2R hens and in 13 weeks of age it was higher than that of the 2R hens. At 13 week of age the mean body weight ± SEM in distinct treatments was as follows: AL 3561 ± 130 g, QR 2710 ± 199 g, 2R 2546 ± 47 g and R 1558 ± 24 g (Fig. 1A). The results of the ANOVA showed significant effect of age ($p < 0.001$), feeding treatment ($p < 0.001$) and their interaction ($p < 0.001$) on body weight.

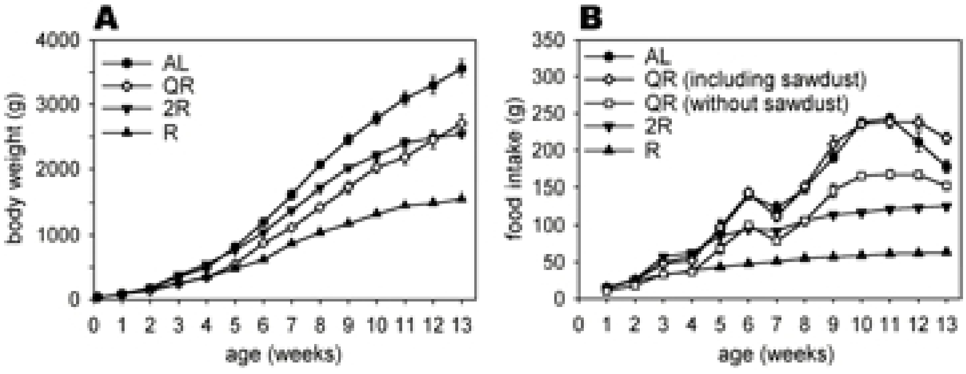

Fig. 1. Mean body weight (A) and food intake (B) at different ages of hens fed on the restricted ration recommended by the breeding company (R), twice that amount (2R), fed by the diet diluted with 30 % sawdust (QR) and *ad libitum* (AL). n = 6 per treatment

Table 1
Significance (*p*-levels) of the effects of testing week (W), time of day (T), feeding treatment (F) and their interactions on proportion of time spent by distinct behaviours. Results from three-way ANOVA.

	Main effects			Interactions			
	W[1]	T[2]	F[3]	W × T	W × F	T × F	W × T × F
Pecking at feeder	n.s.	0.001	0.001	n.s.	n.s.	0.001	n.s.
Drinker directed activity	n.s.	n.s.	0.001	n.s.	n.s.	0.05	n.s.
Object pecking	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sitting	n.s.	0.001	0.001	n.s.	n.s.	n.s.	n.s.
Standing	n.s.	0.001	0.001	n.s.	n.s.	0.001	n.s.
Pacing	0.05	0.05	0.01	n.s.	n.s.	n.s.	n.s.
Preening	n.s.	0.001	0.01	n.s.	n.s.	0.05	n.s.

[1] behavioural tests at 11, 12 and 13 weeks of age
[2] 30 min behavioural tests commencing at 9.30, 13.30 and 17.30
[3] four feeding treatments – AL, QR, 2R and R – see Materials and Methods for details

Figure 1B illustrates food intake in distinct feeding treatments. Starting from 2 and 5 weeks of age R and 2R hens, respectively, consumed the whole amount of food provided in the daily meal. Food consumption curve of QR hens (food mash + sawdust) with free access to food followed the food consumption curve of AL hens. Drop in the food intake at the age of seven weeks in AL and QR hens and to a lesser extent in 2R hens was connected with the change from group to individual housing as well as with the change from starter to grower diet (Fig. 1B).

Statistical analysis of behavioural data (Table 1) proved that there was no significant effect of the testing week on behaviour (except pacing), i.e. behaviour of hens over the three testing weeks was stable and therefore we presented it in Fig. 2 as the means of the observations during consecutive 3 weeks (week 11 to 13). On the other hand, there was highly significant effect of the time of day on behaviours (except drinker directed activity and object pecking) and feeding treatment on behaviours (except object pecking). Out of the interactions the only significant was the effect of an interaction between the time of day and feeding treatment in case of pecking at feeder, drinker directed activity, standing and preening (Table 1), indicating different distribution of behaviours during the day in hens belonging to distinct feeding treatments. There were no intensive changes in behaviour of hens with free access to food (AL and QR) related to the time of day (Fig. 2). In contrast, there was marked diurnal variation in behaviour of quantitatively restricted hens. During the first observation period (09.30-10.00) there were still remains of the food mash in feeders of the 2R and R hens. However, during the second and third observation period the feeders were already empty, but pecking at (empty) feeder only gradually decreased. Although there were differences in time course of the proportion of time spent by pecking at feeder (full and empty combined) between QR and R hens (Fig. 2), sum of the occurrence of this behaviour over the whole day in R hens, as well as in QR ones, was higher in comparison with both AL and 2R hens (R > AL, $p < 0.001$; R > 2R, $p < 0.01$; QR >AL, $p < 0.001$; QR >2R, $p < 0.05$). The whole day sum of drinker directed activity, i.e. drinking and drinker manipulation, was higher in birds with free access to food in comparison with those provided with one restricted daily meal (AL > 2R, $p < 0.001$; AL > R, $p < 0.01$; QR > 2R, $p < 0.001$; QR > R, $p < 0.001$). There was less sitting observed in hens with single restricted daily meal as compared to those with free access to food (R < AL, $p < 0.001$; 2R < AL, $p < 0.001$; R < QR, $p < 0.001$; 2R < QR, $p < 0.05$). There was also difference between more and less intensive quantitative food restriction in proportion of time spent sitting (R < 2R; $p < 0.05$). There was more standing (mainly at the end of day) in quantitatively restricted hens than in those with free access to

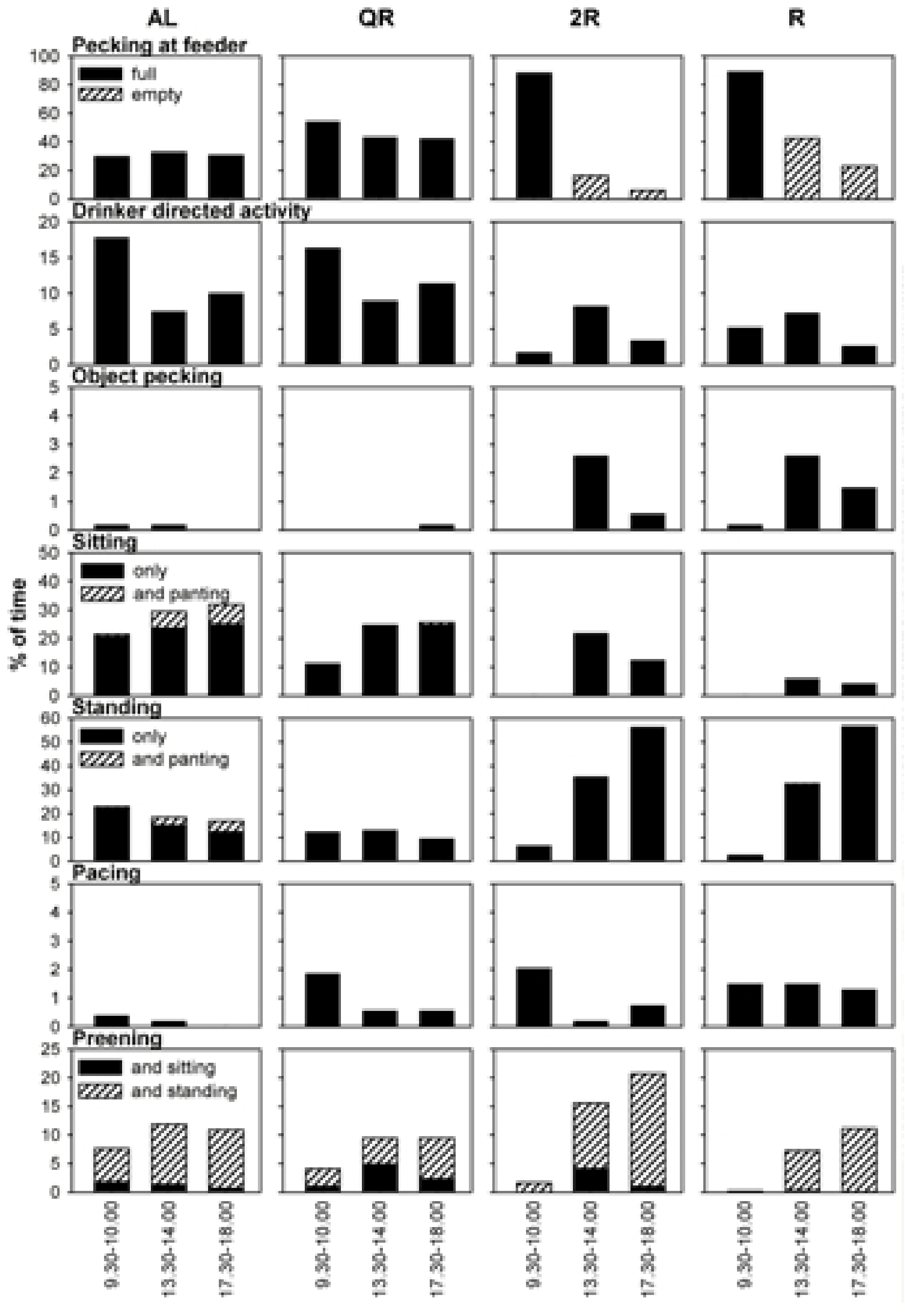

Fig. 2. Mean proportions of time spent in different activities during 30 min commencing at 9.30, 13.30 and 17.30. Raws represent distinct behaviours and columns 4 feeding treatments (AL, QR, 2R, R – see Fig. 1 for details). Values represent mean of three observations in 11, 12 and 13 weeks of age; n = 6 per treatment.

food (R > AL, $p < 0.001$; 2R > AL, $p < 0.001$; R > QR, $p < 0.001$; 2R > QR, $p < 0.001$), and in AL as compared to QR ones (AL > QR, $p < 0.01$). Proportion of time spent pacing was in all food restriction treatments higher than in AL hens (R >AL, $p < 0.001$; 2R > AL, $p < 0.05$; QR > AL, $p < 0.05$). There was less preening in R hens in comparison with AL and 2R ones (R < AL, $p < 0.05$; R < 2R, $p < 0.001$) and less preening in QR hens than in 2R ones (QR < 2R, $p < 0.01$).

Plasma concentration of corticosterone (Fig. 3A) in R hens was significantly higher in comparison with all other treatments (R > AL, $p < 0.001$; R > QR, $p < 0.01$; R > 2R, $p < 0.001$). While the increase in corticosterone level in 2R hens as compared to AL ones did not reach the level of statistical significance, the increase in QR hens in comparison with AL ones was significant (QR > AL, $p < 0.05$). Plasma concentrations of T_3 (Fig. 3B) was in all restricted-fed treatments significantly lower in comparison with AL treatment (QR < AL, $p < 0.01$; 2R< AL, $p < 0.01$; R < AL, $p < 0.001$). In case of plasma concentration of T_4 (Fig. 3C) there was a trend towards increase in QR and 2R hens and significant increase in case of R hens in comparison with AL control (R > AL, $p < 0.01$).

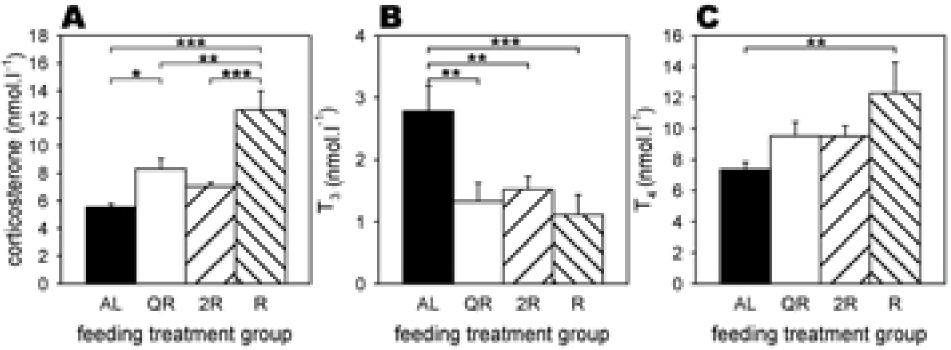

Fig. 3. Plasma concentrations of corticosterone (A), T_3 (B) and T_4 (C) in hens belonging to 4 feeding treatments (see Fig. 1). n = 6 per group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The protein content and glucose concentration in plasma were not changed in hens exposed to qualitative and quantitative food restriction (Fig. 4A, 4D). The uric acid content in plasma of the QR hens was significantly lower in comparison with all other feeding treatments (QR < AL, $p < 0.05$; QR < 2R, $p < 0.05$; QR < R, $p < 0.05$) (Fig. 4B). Food restriction caused elevation of creatinine concentration in 2R hens in comparison with AL and QR hens (2R > AL, $p < 0.05$; 2R > QR, $p < 0.01$) (Fig. 4C). The triacylglycerols levels decreased significantly in QR hens in comparison with AL (QR < AL, $p < 0.05$), in R hens compared to all other treatments (R < AL, $p < 0.001$; R < QR, $p < 0.05$; R < 2R, $p < 0.01$) and there was a trend towards decrease in 2R hens as compared to AL ones (Fig. 4E). On the other side cholesterol levels (Fig. 4F) in plasma of hens from all restricted treatments were significantly increased in comparison with the AL hens (QR > AL, $p < 0.05$; 2R > AL, $p < 0.05$; R > AL, $p < 0.01$).

Discussion

As a result of chronic quantitative and qualitative food restriction at 13 weeks of age the body weights of R, 2R and QR hens were 0.4, 0.7 and 0.8 fold of AL control. These values as well as food consumption correspond with values reported by other authors in AL, 2R and R broiler breeder chickens (Bruggeman et al. 1998, 1999; Savory et al. 1993, 1996). The

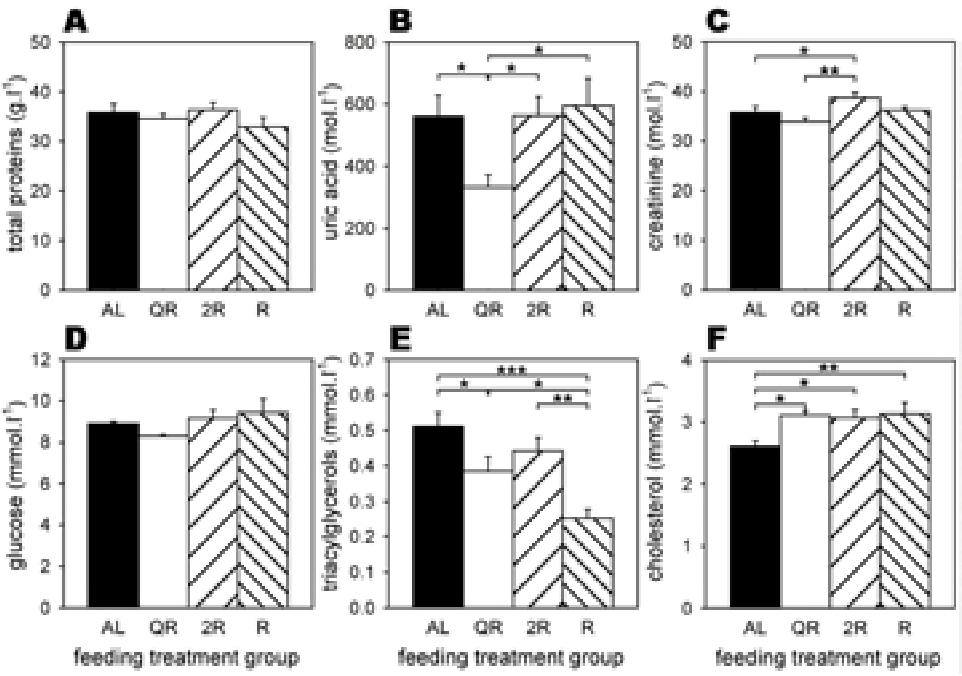

Fig. 4. Total proteins in plasma (A) and plasma concentrations of uric acid (B), creatinine (C), glucose (D), triacylglycerols (E) and cholesterol (F) in hens belonging to 4 feeding treatments (see Fig. 1). n = 6 per group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

dilution of diet by addition of 50 % sawdust in the study of Savory et al. (1996) reduced the body weight of chickens to the level lower than in R hens.

Vilariño et al. (1996) showed that time spent by chickens at the trough is affected by the form of food. Hens fed with the pelleted diets spent considerably shorter time at the trough than hens fed with mash. That explains why restricted-fed (R) broiler breeders Ross consumed their daily ration in less than 15 min, when provided as pellets (Savory and Maros 1993; Košťál and Savory 1994), while in present experiment under comparable conditions it took them more than 60 min to consume the same amount of food mash.

Marked diurnal variation in behaviour of hens belonging to both quantitatively restricted treatments (R and 2R) in present experiment was characterised by decreasing proportion of time spent pecking at feeder and increasing proportion of time spent standing and preening throughout the day. Similar distribution of behaviours during the day in quantitative restricted broiler breeders were reported earlier (Košťál et al. 1992; Savory and Maros 1993; Savory et al. 1996; Savory and Košťál 1996). Minor differences in frequences of behaviours could be attributed to the differences in age and form of food (pellets vs. mash).

Diurnal distribution of activities of QR hens the age of 11 – 13 weeks had similar development to that of AL treatment. In QR hens there was reduced amount of oral stereotypies (pecking at objects resp. empty feeder) as compared to R and 2R hens. On the other side QR hens were more active than AL ones and spent more time by food intake and pacing. Mash diet, in particular when it is diluted, makes it difficult for hens to satisfy their nutrient requirements. According to Vilariño et al. (1996) this induces a mild frustration state in the hen, expressed by numerous visits to the trough over a longer period of time and higher frequency of trough-oriented pecking behaviour. Peletting of diluted diet seems to limit the excessive trough-oriented behaviour and permit the feeding of such diet with no adverse effects on birds' behaviour (Vilariño et al. 1996).

Chronic food restriction represents for organism permanent stress and, as a result, whole spectrum of metabolic processes including a shift from anabolism to catabolism, from lipogenesis to lipolysis, occurs. Adaptive changes are mediated by many metabolic hormones. Data obtained in chickens show that food restriction modifies the plasma levels of hormones involved in energy metabolism and growth processes, such as T_3, T_4, GH, IGF-I (Darras et al. 1995; Buyse et al. 2000) and corticosterone (Savory et al. 1996; Hocking et al. 1998, Carsia and Weber 2000).

In our experiment, the significant increase of corticosterone levels in immature (13 weeks old) broiler breeder hens subjected to intensive (recommended by the breeding company) quantitative food restriction was found. Less severe restriction (2R) did not cause corticosterone elevation in comparison with AL. These results are in agreement with results previously obtained by Savory et al. (1996) and Savory and Mann (1997) using similar experimental design. Qualitative food restriction using diet diluted by 30 % of hardwood sawdust in immature broiler breeder hens in this study did not prove the welfare benefits of this procedure. There is an evidence of stress in QR hens. Although the plasma concentration of corticosterone in QR hens was significantly lower than that in R hens, it was still significantly higher than in AL hens. Savory et al. (1996) found in hens fed by diet diluted with 50 % of softwood sawdust plasma corticosterone levels substantially higher than in R hens. Much milder qualitative restriction applied here still caused an elevation of corticosterone in comparison with AL hens, although the body weight of these hens was at 13 week comparable with 2R hens, in which no significant elevation of plasma corticosterone was detected.

Our results showed different response of T_3 and T_4 plasma concentrations to food restriction. While T_3 decreased in all restricted treatments, T_4 increased significantly in R hens and there was a marked trend towards increase in QR and 2R hens, all in comparison with the AL hens. These findings are consistent with data from previous studies (Klandorf and Harvey 1985; Newcombe et al. 1992; Bruggeman et al. 1997; Gonzales et al. 1998; Dewil et al. 1999). The most detailed study in broiler breeder hens by Bruggeman et al. (1997) showed, that during ontogeny (2 – 24 weeks) T_3 concentration decreased and T_4 concentration increased in both restricted and *ad libitum* fed hens. However, a nutrition effect is more marked than the age effect (Bruggeman et al., 1997). The nutritional stimuli are supposed to influence control of thyroid function (Klandorf and Harvey 1985).

There were no significant differences between feeding treatments in plasma glucose or total protein levels found in present experiment. Dewil et al. (1999) described decrease in plasma glucose induced by the short food restriction (1 day) in chickens. However, glucose returned to normal levels, i.e. similar to those recorded in *ad libitum* fed hens, after a 7 day restriction. This was interpreted in terms of the effectiveness of the glucose homeostatic mechanisms (Dewil et al. 1999). In agreement with this assumption are findings of Rosebrough et al. (1992, 1999), who did not find altered plasma glucose concentrations in male broiler chickens neither in groups with increasing dietary energy : protein ratio nor after addition of the 1 mg of T_3 per kg of diet.

There is controversy in data concerning relation between dietary protein intake and plasma uric acid levels. Hevia and Clifford (1977) reported a positive linear correlation between plasma uric acid and dietary protein intake. On the other hand Rosebrough et al. (1992, 1999) found in male broiler chickens that decreasing dietary energy: protein ratio increased plasma uric acid concentration and addition of T_3 into diet decreased uric acid concentration. In present experiment only in QR hens concentration of uric acid was significantly lower as compared to all other treatments.

In present experiment plasma concentration of triacylglycerols was decreased in R and QR 13 weeks old broiler breeder hens compared with AL ones. Hocking et al. (1994) reported lower plasma triacylglycerols in one-year-old genetically fat and lean broiler breeder females subjected to food restriction. Rosebrough et al. (1992, 1999) found increasing plasma triacylglycerols with increasing dietary energy : protein ratio as well as with an addition of T_3 into the diet in male broiler chickens. An association between T_3 and triacylglycerols is in accordance with our finding that both plasma T_3 and triacylglycerols concentration decreased in a similar manner with increasing food restriction.

Studies of models of physiological stress in chickens using the treatment with ACTH or corticosterone showed that the metabolic changes associated with stress in chicken are increased plasma glucose, total proteins, triacylglyceroles and cholesterol (Davison et al. 1983; Latour et al. 1996; Puvadolpirod and Thaxton 2000 ab). Response in these metabolic parameters to chronic food restriction differ in several respects. However, increased plasma cholesterol characterise both models.

In summary, there were the signs of reduced welfare detected in hens fed according to the restriction programme recommended by the breeding company. Although the behaviour of qualitative restricted hens reminds more the behaviour of *ad libitum* fed hens than that of the quantitative restricted ones, it was not associated with reduction in blood indices of stress and so cannot be taken as indicator of improved welfare.

Vplyv reštrikcie krmiva na správanie, endokrinné a metabolické ukazovatele rodičov broilerov

Sledovali sme vplyv reštrikcie krmiva na správanie, endokrinné a metabolické parametre u 13 týždňových sliepočiek materskej línie kúr mäsového typu (brojlerov). Sliepočky kŕmené *ad libitum* sme porovnávali s jednou kvalitatívne reštringovanou skupinou (krmivo zriedené 30 % pilín z tvrdého dreva) a s dvoma kvantitatívne reštringovanými skupinami (denná dávka doporučená šľachtiteľskou firmou a dvojnásobok tohoto množstva). V správaní kvantitatívne reštringovaných sliepočiek ktoré dostávali jednu dennú kŕmnu dávku boli výrazné diurnálne zmeny, zatiaľ čo prvky správania u sliepočiek s voľným prístupom ku krmivu (*ad libitum* kŕmené a kvalitatívne reštringované) boli rovnomernejšie distribuované počas dňa. Skupina s intenzívnou kvantitatívnou reštrikciou krmiva mala zvýšenú hladinu plazmatického kortikosterónu. Hoci zvýšenie koncentrácie kortikosterónu u skupiny s kvalitatívnou reštrikciou bolo nižšie než u skupiny s kvantitatívnou reštrikciou, bolo v porovnaní s *ad libitum* kontrolou signifikantné, čo naznačuje, že aj mierna kvalitatívna reštrikcia krmiva je stresujúca. V porovnaní s *ad libitum* kŕmenými sliepočkami bola u všetkých typov reštrikcie krmiva znížená hladina plazmatického T_3, zatiaľ čo plazmatický T_4 bol vplyvom intenzívnej kvantitatívnej reštrikcie zvýšený. Reštrikcia krmiva neovplyvnila koncentráciu plazmatickej glukózy ani celkových bielkovín, ale hladina triacylglycerolov bola znížená a cholesterolu zvýšená ako dôsledok reštrikcie. Koncentrácia kreatinínu bola zvýšená u sliepočiek s miernejšou formou kvantitatívnej reštrikcie a koncentrácia kyseliny močovej znížená u kvalitatívne reštringovaných sliepočiek v porovnaní s ostatnými skupinami. Tieto výsledky ukazujú, že hoci správanie kvalitatívne reštrikčne kŕmených pripomína viac správanie *ad libitum* kŕmených sliepočiek, ich fyziologický stav je podobný viac kvantitatívne reštringovaným sliepočkám.

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