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The effect of feed restriction and housing system on performance, organ proportion and microbiota

*Jan Tyl[1,2], Eva Tůmová[2], Darina Chodová[2]**

[1] *International Poultry Testing Station Ústrašice, Czech Republic*
[2] *Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic*

**Corresponding author: chodova@af.czu.cz*

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Abstract: The aim of the present study was to evaluate the effects of feed restriction and the combination of feed restriction with pasture on the performance parameters of fast-growing chickens, the proportion of internal organs and the caecum microbiota. In the experiment, one-day-old Ross 308 chickens were divided into three groups. Group 1 was fed *ad libitum* (AL), group 2 was restricted on feed at a rate of 70% *ad libitum* (R), group 3 was restricted to the same age and level as group 2, and was restricted to 22 days on pasture (FR). Feed restriction and the combination of feed restriction and pasture significantly reduced body weight beginning at the age of 14 days and at the end of the experiment (at 35 days), mortality, and the European Production Efficiency Factor (EPEF) but improved the feed conversion ratio (FCR) beginning at the 4th week of age. Conversely, feed restriction and the combination of feed restriction and pasture significantly increased the proportion of liver, gizzard and *Lactobacillus* in the caecum. In summary, feed restriction and the combination of feed restriction in the free range had negative effects on growth, feed conversion ratio and economic profit, presumably because of the short realimentation period and because pasture did not have a beneficial effect.

Keywords: chicken; limited feeding; free range; growth

Various strategies, including innovative feed supplements, nutrition and feed management systems, and grazing management using regenerative farming techniques, are needed to reduce livestock greenhouse gas emissions (Park 2022). Chicken meat production is assumed to meet al. these challenges. However, intensive chicken meat production is facing challenges such as the maintenance of rentability and the interest of customers in meat quality. Meat quality is affected by many factors, among which feeding plays an important role. Different feeding strategies can be applied, including feed restriction, nutrient reduction, alternative protein sources and pasture. One feeding strategy is restriction of feed. Feed restriction improves nutrient utilisation, leading to compensatory growth in the realimentation period. Previous studies confirmed that compensatory growth followed restriction in chickens (Tumova et al. 2002; Lunedo et al. 2019), but the results depend on the intensity and duration of the restriction. Feed restriction programmes are usually applied in the

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early life of broilers to induce compensatory growth (Tumova et al. 2022), stimulating feed efficiency by decreasing the FCR (Gratta et al. 2019) and reducing the mortality rate (Mohammadalipour et al. 2017). Studies have shown that a restriction regimen results in increased crop, proventriculus and gizzard weights to maintain feed for a longer time than does *ad libitum* (ADL) feeding, which may be involved in enhancing feed utilisation (Fondevila et al. 2020). Feed restriction influences the composition of the microbiota in the intestines, for example, increasing *Lactobacilleceae* in the ileum and caecum (Metzler-Zebeli et al. 2019). Moreover, *Lactobacillus* species produce lactic acid, which suppresses pathogen proliferation and adhesion, leading to stabilisation of the gut microbial ecosystem (Ebeid et al. 2022).

A free-range housing system for chicken meat production improves natural behaviour and may provide a source of energy, amino acids, and bioactive compounds (Sales 2014). Englmaierova et al. (2021) reported that pasture access increased final body weight, increased breast percentage and positively affected meat quality. Outdoor access leads to richer and more complex microbiota (Varriale et al. 2022). The effect of pasture is affected by its intake, and pasture consumption can be increased by feed restriction (Ponte et al. 2008; Englmaierova et al. 2021). Data on the effects of feed restriction and pasture conditions were obtained for medium-growing chickens, but data for fast-growing chickens were unavailable. Therefore, the present study aimed to evaluate the effects of *ad libitum* feeding, feed restriction and feed restriction in the free range on growth, the size of internal organs and the intestinal microbiota.

MATERIAL AND METHODS

The experiment was performed at the International Poultry Testing Station in Ústrašice in accordance with valid legislative rules and approved by the Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic.

One-day-old Ross 308 chickens were used in the present study. In total, 1 260 birds (1 : 1 sex ratio) were divided into three groups. Group 1 was fed *ad libitum* (AL) during the whole experiment; group 2 was restricted to between eight and 14 days of age at a rate of 70% *ad libitum* (R) and then fed *ad libitum* until the end of the experiment. The third group was restricted to the same age as group 2 and to 22 days of age in combination with free-range housing (FR). In each group, three replications were used. Indoor chickens were housed in pens with wooden bedding, the density was 14 birds/m^2, and the free-range group was 22 days old. The chickens were placed under the shelter in outdoor pens with 4 m^2 pasture space per bird. The housing conditions agreed with the recommended standards for Ross 308 chickens. Chickens received three feed mixtures – the starter mixture until 14 days, the grower mixture from 15 to 28 days and the finisher mixture from 29 to 35 days of age – when the experiment was finished. The compositions of the feed mixtures are given in Table 1. Water was provided *ad libitum* for all groups during the whole experiment. The lighting regime consisted of 23 h of light on days 1 to 7 h and 18 h of light from day 8 until the end of the experiment. Group 3 was subjected to a natural light regime in the free-range period.

In the experiment, birds were individually weighed beginning on day one at one-week intervals. Feed consumption was recorded weekly for each pen. The data were used to calculate the feed conver-

Table 1. Composition and calculated nutrient content in feed mixtures

Item	Starter	Grower	Finisher
Wheat (%)	46.2	59.5	64.1
Maise (%)	15.0	8.0	5.0
Soyabean meal (%)	30.6	25.7	22.8
Fish meal (%)	1.0	–	–
Monocalcium phosphate (%)	0.53	0.34	0.17
Limestone (%)	1.4	1.2	1.1
Salt (%)	0.23	0.20	0.23
Soyabean oil (%)	3.0	1.0	–
Animal fat (%)	–	2.4	5.3
Sodium sulfate (%)	0.11	0.12	0.08
Premix of amino acids (%)	0.75	0.76	0.69
Premix of vitamins (%)	0.94	0.72	0.41
Calculated nutrient content			
Crude protein (g/kg)	216.3	195.9	184.8
Crude fat (g/kg)	51.0	52.7	70.8
Lysin dig. (g/kg)	11.8	10.6	9.4
Methionine (g/kg)	5.6	4.9	4.3
Metabolisable energy (MJ/kg)	12.5	12.8	13.4

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sion ratio (FCR). Mortality was recorded every day. The experimental economic efficiency was evaluated by the European performance efficiency factor (EPEF) using the following formula:

$$EPEF = (FLW \times VIA)/(LFP \times FCR) \times 100 \quad (1)$$

EPEF – the European performance efficiency factor;
FLW – final live weight (kg);
VIA – viability (%);
LFP – length of fattening period (days);
FCR – feed conversion ratio (kg).

At the age of 35 days, 10 cockerels per group on average weight were selected for slaughter analysis. Chickens were eviscerated immediately after slaughtering, and the weights of the internal organs were used for calculating the proportions of intestines, livers, gizzards and heart percentages relative to the carcass weight. In the slaughtered cockerels, the caecum was taken for microbiota analysis. For assessment of intestinal microbiota, total counts of anaerobic bacteria (TCs), as well as counts of bifidobacteria, lactobacilli and *Escherichia coli*, were determined by the plate-count method using a tenfold dilution of each sample up to 10^{-9}. Prior to the analysis, after slaughtering, the samples from the caecum of each killed chicken were collected immediately and transferred aseptically into CO_2^-flushed sterile tubes containing Nutrient Broth No. 2 (5 g/l; procured from Oxoid, Basingstoke, UK), tryptone (5 g/l; Oxoid, Basingstoke, UK), yeast extract (2.5 g/l; Oxoid, Basingstoke, UK), Tween 80 (0.5 ml/l; Sigma-Aldrich, Burlington, MA, USA), and L-cysteine (0.25 g/l; Sigma-Aldrich, Burlington, MA, USA). To determine the total anaerobe composition, Wilkins-Chalgren anaerobic agar (43 g/l; Oxoid, Basingstoke, UK) supplemented with veganetone soya peptone (5 g/l; Oxoid, Basingstoke, UK), L-cysteine (0.5 g/l; Sigma-Aldrich, Burlington, MA, USA), and Tween 80 (1 ml/l; Sigma-Aldrich, Burlington, MA, USA) was used. The same media were supplemented with the antibiotics mupirocin (100 mg/l; Oxoid, Basingstoke, UK) and norfloxacin (1 000 mg/l; Oxoid, Basingstoke, UK), and glacial acetic acid (1 ml/l) was used for the determination of bifidobacteria. Culture plates for the growth of anaerobes and bifidobacteria were incubated in anaerobic jars (Anaerobic Plus System; Oxoid, Basingstoke, UK) at 37 °C for 48 h. Lactobacilli were cultured using Rogosa Agar (82 g/l; Oxoid, Basingstoke, UK) adjusted to pH 5.4 by glacial acetic acid for 48 h under microaerophilic conditions. *E. coli* counts were determined using TBX medium (36.6 g/l; Oxoid, Basingstoke, UK) by incubating the plates aerobically at 37 °C for 24 h (Verhaegen et al. 2015). Total counts of anaerobes and bifidobacteria were cultured using the pour-plate method, lactobacilli were cultured using the double-layered pour-plate method, and *E. coli* was cultured using the spread-plate method.

The results of the experiment were analysed by one-way analysis of variance (ANOVA) in SAS software v9.4 (SAS Institute, Inc., Cary, NC, USA). The significance of the differences in growth was determined by the Scheffe test, and the differences in feed consumption, organ percentage and microbiota composition were also determined by the Duncan test. $P \leq 0.05$ was considered to indicate statistical significance, and the data in the row are indicated by different superscripts.

RESULTS AND DISCUSSION

The growth of the chickens (Table 2) was similar among the groups until the end of feed restriction at 14 days of age. At this age, the live weight (LW) of the restricted chickens was significantly lower, approximately 26% and 23% lower than that of the AL group. The greatest difference between the restricted and AL chickens was observed at 21 days of age ($P = 0.001$), when the LW of chickens in both restricted groups was less than 30%. Following this age, the growth of the restricted chickens slightly increased, and at the end of the experiment, the growth of the LW group was approximately 25% lower than that of the AL group ($P = 0.001$). There were no significant differences in LW between indoor and free-range chickens. The results showed that the growth depression of the restricted chickens was most significant at 21 days, one week after restriction, and at a similar level. Then, growth negligibly increased, but the chickens could not compensate for their growth reduction, presumably due to the short realimentation period, which corresponds with the findings of Lunedo et al. (2019) and Tumova et al. (2021; 2022). Neither free range nor access to pasture had a positive effect on growth; these findings disagree with those of Ponte et al. (2008), and Englmaierova

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Table 2. The effect of feeding regime and pasture on performance

Measurement	Group			SEM	Significance
	AL	R	FR		
Live weight at 1 day of age (g)	44.3	43.9	44.1	0.130	0.699
Live weight at 7 day of age (g)	192	196	185	2.245	0.135
Live weight at 14 day of age (g)	559[a]	417[b]	429[b]	2.732	0.001
Live weight at 21 day of age (g)	1 056[a]	723[b]	733[b]	5.207	0.001
Live weight at 28 day of age (g)	1 469[a]	1 104[b]	1 137[b]	6.442	0.001
Live weight at 35 day of age (g)	2 034[a]	1 520[b]	1 534[b]	9.815	0.001
FCR 1st week (kg)	1.46	1.72	1.76	0.085	0.345
FCR 2nd week (kg)	1.11	1.19	1.13	0.026	0.503
FCR 3rd week (kg)	1.42	1.46	1.48	0.040	0.893
FCR 4th week (kg)	2.22[a]	1.69[b]	1.60[b]	0.108	0.007
FCR 5th week (kg)	2.05[a]	1.71[b]	1.80[b]	0.103	0.003
Mortality 1–35 days (%)	2.14[a]	0.48[b]	0.24[b]		0.001
EPEF	343[a]	280[b]	288[b]	2.610	0.001

AL = *ad libitum*; EPEF = European production efficiency factor; FCR = feed conversion ratio; FR = free range; R = restriction
[a,b]$P \leq 0.05$

et al. (2021), but both studies were performed on medium-growing chickens where chickens had longer realimentation periods.

The growth of chickens also depends on feed consumption, and in the present study, the FCR was not affected until three weeks (Table 2). In the 4th and 5th weeks, the experimental groups had significantly lower FCRs than the AL group. It is assumed that lower feed consumption was a reason for the lack of compensatory growth. Tumova and Chodova (2018) and Tumova et al. (2021; 2022) did not observe an effect of feed restriction on the FCR. Ebeid et al. (2022) postulated that the decreased growth and feed consumption of restricted chickens might be attributed to a reduction in essential amino acids, which inhibits protein synthesis and increases proteolysis. In addition, pasture access did not have a beneficial effect on the FCR, according to Ponte et al. (2008). Feed restriction led to a reduction in mortality, which was only 22% in group 2 and 11% in group 3 in the free-range group compared to that in the AL group; these findings are consistent with those of Mohammadalipour et al. (2017), Tumova and Chodova (2018) and Tumova et al. (2019). The decreased mortality of restricted chickens is due to a decreased incidence of sudden death syndrome and ascites (Lippens et al. 2000; Mohammadalipour et al. 2017; Tumova et al. 2019) and leg problems (Lippens et al. 2000). The economic effect of the evaluated factors was evaluated via the EPEF. In both restricted groups, the trait density was significantly lower than in the AL group and reached 82% and 84%, respectively. A lower economic status was associated with a 25% lower final live weight and better FCR; mainly, low mortality could not eliminate the slow growth of the restricted chickens. Delezie et al. (2010) and Tumova et al. (2021) also described a negative effect of feed restriction on production effectiveness.

Internal organs play a vital role in body development and might be affected by the feeding regime because of early development. The feeding regime had a minor effect on the intestinal proportion at the end of the experiment (Table 3). However, the gizzard proportion was significantly greater in the restricted chickens than in the control chickens, with no differences between the indoor and free-range groups; however, in the free-range group, the proportion tended to increase and might be associated with pasture consumption. Tumova and Chodova (2018) reported that feed restriction had a positive effect on gizzard development and that growth increased during the last week of the fattening period. In the present study, a greater proportion of gizzards at the end of the experiment indicated greater feed consumption, which tended to compensate for growth because,

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Table 3. The effect of feeding regime and pasture on internal organ development

Measurement	Group			SEM	Significance
	AL	R	FR		
Slaughter weight (g)	2 201[a]	1 600[b]	1 599[b]	54.90	0.001
Intestine proportion (%)	8.61	9.76	8.85	0.282	0.221
Gizzard proportion (%)	1.69[b]	2.22[a]	2.35[a]	0.088	0.002
Liver proportion (%)	2.87[b]	3.10[a]	3.28[a]	0.069	0.050
Heard proportion (%)	0.68	0.75	0.82	0.025	0.072

AL = *ad libitum*; FR = free range; R = restriction
[a,b]$P \leq 0.05$

in the last week, the differences between the AL and control groups decreased and showed that the realimentation period was short enough to allow complete compensation of growth. Like those in the gizzard group, the liver proportion in the restricted group increased ($P = 0.05$), and neither of the restricted groups significantly differed. Tumova et al. (2019) observed a greater liver proportion and postulated that this increase is related to greater functional activity and glycogen storage and therefore to increased body fat deposition (Tumova et al. 2019).

In contrast, Tumova and Chodova (2018) did not detect differences in liver weight between ALs and feed-restricted chickens, and this disproportionate difference can be explained by the findings of Govaerts et al. (2000), indicating that liver development is affected by the intensity of feed restriction and that less severe restriction does not cause liver breakdown. The feeding regime did not significantly affect the hearing distribution, but it tended to be greater in the restricted groups than in the AL group. Similar results were observed by van der Klein et al. (2017), Tumova and Chodova (2018), Tumova et al. (2019), and Mohammadalipour et al. (2017), who suggested that higher heart mass increases the oxygen supply for metabolism.

The present study evaluated the caecal microbiota, total number of bacteria, total number of *E. coli* and total bacteria, and the total number of *Lactobacillus* and *Bifidobacterium* bacteria (Table 4). Only *Lactobacillus* spp. were significantly affected by feeding regime and housing. The highest *Lactobacillus* count ($P = 0.002$) was observed in restricted group 2, which was significantly greater than that in the restricted group, which was in the free range and was significantly lower than that in the AL group. These results correspond with Metzler-Zebeli et al. (2019) and Ebeid et al. (2022). The proliferation of *Lactobacillus* is associated with increased lactic acid production, which suppresses pathogen proliferation (Ebeid et al. 2022). In contrast with the findings of Varriale et al. (2022), free-range housing did not positively affect the gut microbiota; however, the authors postulated that the results of the effect of environmental factors have limitations and should be interpreted cautiously.

In conclusion, both experimental groups exhibited worse performance. Live weight was reduced

Table 4. The effect of feeding regime and pasture on intestinal microbiota

Measurement	Group			SEM	Significance
	AL	R	FR		
Total count of bacteria	9.51	9.44	9.43	0.039 3	0.677
Escherichia coli	7.27	6.96	6.56	0.136	0.095
Coli total	7.36	6.99	6.59	0.136	0.063
Lactobacillus	7.62[c]	8.89[a]	8.28[b]	0.142	0.002
Bifidobacteria	7.51	7.32	8.06	0.218	0.368

AL = *ad libitum*; FR = free range; R = restriction
[a–c]$P \leq 0.05$

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after the end of restriction, and chickens could not compensate for their feed limitations, presumably due to low feed consumption. However, in the last week of the experiment, the growth of the restricted chickens slightly increased. A positive effect of FR and the combination of FR and pasture was detected for mortality. A greater proportion of gizzards and livers might support compensatory growth. However, in the present study, the realimentation period was too short, and chickens in the free range consumed a low amount of pasture water to improve the results. The feeding regime had a minor effect on the caecal microbiota. However, further studies are needed to determine the mechanism underlying the effects of feed restriction and pasture.

Conflict of interest

The authors declare no conflict of interest.

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