Changes of haematological and biochemical indices with age in rabbits with *ad libitum* and limited feed intake

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Abstract

The aim of this study was to examine the effects of a one-week feed restriction of different intensities on haematological and biochemical blood indices at the end of the feed restriction and during the realimentation period in growing rabbits. Hyplus rabbits weaned at 35 days of age (n = 195) were divided into three groups: rabbits fed *ad libitum* (AL) and groups with reduced feed of 50 g (R50) or 65 g (R65) per rabbit per day from 42 to 49 days of age. At the end of the feed restriction and in the following period, haematological and biochemical blood indices were measured at weekly intervals. The live weight at 70 days of age was lower ($P \leq 0.001$) in restricted rabbits (–14.5% and –15.5% for group R50 and R65, respectively, compared to AL). Feed restriction significantly ($P \leq 0.011$) decreased the number of erythrocytes (–6.2% and –7.4% for group R50 and R65, respectively, compared to AL) and haemoglobin content ($P \leq 0.008$). The mean corpuscular volume increased ($P \leq 0.033$) in restricted rabbits. Interaction of the feeding regime and age ($P \leq 0.007$) was observed in cholesterol with the highest concentration in R50 at 56 days (4.41 mmol/l) and the lowest in R65 at 70 days and in AL at 63 days of age (1.30 mmol/l). The concentration of non-esterified fatty acids ($P \leq 0.003$) and triacylglycerides ($P \leq 0.048$) were significantly lower in restricted rabbits. It could be concluded that feed restriction influenced mainly erythrocytes and haemoglobin. The changes in biochemical blood characteristics suggest the restriction did not affect protein metabolism, however, results indicate lipid depletion in restricted rabbits.

Hyplus, feed restriction, blood, metabolic characteristic

Limited feed intake is applied in growing rabbits as a prevention of digestive disorders around the post-weaning age (Di Meo et al. 2007). It has been reported to improve feed efficiency (Tůmová et al. 2003; Di Meo et al. 2007; Gidenne et al. 2009, 2012) and to reduce carcass fat deposition (Gondret et al. 2000; Tůmová et al. 2003, 2007).

Blood characteristics in rabbits are used for clinical diagnosis of organic, infectious and several parasitic diseases, detecting stress conditions, and assessing the metabolic condition of animals (Archetti et al. 2008; Çetin et al. 2009). Haematological and biochemical blood indices are influenced by numerous factors including the breed (Martinec et al. 2012), sex (Çetin et al. 2009), age (Jeklova et al. 2009), diurnal and seasonal variation (Çetin et al. 2009), physiological condition (Wells et al. 1999), and nutrition (Addass et al. 2012). Haematological constituents reflect the physiological responsiveness of the animal to the internal and external conditions which include feeding (Etim et al. 2014). There is a lack data of the effect of the feeding regime on haematology in rabbits. For example, Tůmová et al. (2007) stated that most haematology indices in rabbits were not affected by feed restriction, only the lymphocyte counts were increased and neutrophil counts were reduced in the restricted group. In domestic fowl, haemoglobin was reduced when broilers were exposed to stress caused by feed restriction, whereas the number of erythrocytes increased significantly (Maxwell et al. 1990).

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After the end of the feed restriction, when animals are fed *ad libitum* again, various degrees of compensatory growth can be exhibited in rabbits. These changes in growth during feed restriction and the following realimentation period can be manifested in the proteolytic enzyme function and metabolic processes in the organism represented in changes of biochemical blood indices. There is a lack of information regarding the effect of feed restriction on the biochemical characteristics in rabbits. Van Harten and Cardoso (2010) found that the glucose concentration was not affected by feed restriction in the New Zealand rabbit. Likewise, Sartory et al. (1995) reported that blood glucose concentrations in birds seemed markedly resistant to prolonged feed deprivation. In contrast, Rommers et al. (2004) observed a lower glucose concentration during the period of feed restriction in rabbit does. According to Kersten et al. (1999), fasting decreased adipose tissue lipogenesis and increased the rate of lipolysis. Similarly, van Harten and Cardoso (2010) detected a lower concentration of non-esterified fatty acids (NEFA) and triacylglycerides (TAG) in restricted rabbits. On the other hand, Rommers et al. (2004) observed a similar concentration of NEFA and plasma urea nitrogen in rabbits fed *ad libitum* and restricted rabbits during the time of feed restriction, however, in the following period, these authors detected higher NEFA as well as TAG concentrations in rabbits fed *ad libitum*. Data inconsistencies may be affected by the method of feed restriction, its commencement, and duration.

The aim of the current study was to evaluate the effects of a one-week feed restriction with different intensities on haematological and biochemical indices at the end of the feed restriction and during the realimentation period.

Materials and Methods

The effects of a one-week intensive feed restriction in Hyplus rabbits (PS 19 × PS 59) were studied. The experiment was approved by the Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic. Weaned rabbits with similar live weights at 35 days of age (n = 195) were divided into three experimental groups: rabbits fed *ad libitum* (AL); rabbits with a limited feed intake of 50 g per animal per day from 42 to 49 days of age (R50); and rabbits with a limited feed intake of 65 g per animal per day from 42 to 49 days of age (R65). The rabbits were housed in wire net cages with a stocking density of 3 rabbits per cage (floor density of 0.16 m^2 per rabbit). Before and following the feed restriction, the rabbits were fed *ad libitum*. Water was available *ad libitum* for all groups throughout the whole experiment. Rabbits were fed using a commercial pelleted diet containing 17.1% crude protein, 20.7% crude fibre, and 2.8% ether extract. Environmental conditions were the following: temperature 15–17 °C, relative humidity 55–60% and lighting regime 12 L:12 D.

The live weight was recorded at 35, 42, 49, and 70 days of age. Blood was taken from the jugular vein from eight rabbits per each treatment at the end of the feed restriction at 49 days of age and at weekly intervals. The samples (n = 96) for haematological examination were taken into tubes containing dipotassium salt of ethylenediamine tetraacetic acid (K_2EDTA). Detection of haematological characteristics was carried out in samples stored at 4 °C within 24 h after blood collection. The erythrocyte count, leukocyte count, haemoglobin concentration and haematocrit were detected using an automatic haematological analyser Coulter Model ZF (Coulter Electronics Ltd., England). The leukocyte count was detected by staining using the Pappenheim method. The mean cell volume (MCV) was calculated from the erythrocyte count and haematocrit values.

Samples for biochemical evaluation (n = 96) were collected into tubes without an anticoagulant agent. Blood serum was separated by centrifugation of samples at 1,000 *g* for 10 min and then stored at –70 °C until analysis. Biochemical indices including concentrations of total protein, urea, glucose, cholesterol, NEFA, and TAG were determined using the commercial set Randox (Randox Laboratories Ltd., Crumlin, UK) and using the spectrophotometer Libra S22 (Biochrom Ltd., UK). The values of blood biochemical indices were determined by counting from the absorbance.

The results were processed by two-way analysis of variance ANOVA (with interaction between group and age) using the general linear model (GLM) procedure of statistical analysis system (SAS Institute Inc. 2003). A P value of ≤ 0.05 was considered significant for all measurements.

Results

The live weight of rabbits is shown in Table 1. Live weights of rabbits before feed restriction at 42 days of age did not differ significantly. After the feed restriction at 49 days of age, the live weight decreased significantly ($P \leq 0.001$) in both restricted groups of

Table 1. The effect of one-week feed restriction on live weight in broiler rabbits.

Factor		Live weight at 35 days (g)	Live weight at 42 days (g)	Live weight at 49 days (g)	Live weight at 70 days (g)
Group	AL	1032	1389	1865[a]	3150[a]
	R50	1029	1378	1392[b]	2692[b]
	R65	1025	1355	1383[b]	2662[b]
SEM		12.00	16.02	24.75	47.53
Significance					
Group		0.974	0.675	< 0.001	< 0.001

Different superscripts indicate significant differences (^{a,b} $P \leq 0.05$)
AL - *ad libitum*; R50 - 50 g of feed per rabbit per day; R65 - 65 g of feed per rabbit per day
SEM - standard error of the mean

rabbits compared to the control group. At the end of the experiment, both restricted groups had significantly ($P \leq 0.001$) lower live weights than the group fed *ad libitum* (–14.5% for R50 and –15.5% for R65, compared to AL).

The results of haematological blood indices of restricted rabbits and rabbits fed *ad libitum* are given in Table 2. No interaction between group and age was detected for the haematological indices. The erythrocyte counts were affected by the feed restriction with significantly ($P \leq 0.011$) lower values in both restricted groups compared to the rabbits fed *ad libitum*. The highest differences were detected at 63 and 70 days of age. Erythrocytes in all groups decreased significantly ($P \leq 0.001$) at 56 days, but in following weeks the

Table 2. The effect of one-week feed restriction on haematological indices in broiler rabbits.

Group	Age (days)	Erythrocytes (T/l)	Haemoglobin (g/dl)	Leukocytes (G/l)	Haematocrit (l/l)	MCV (fl)
AL	49	5.80 ± 0.60	12.51 ± 1.29	3.23 ± 1.41	45.38 ± 4.86	78.13 ± 2.59
	56	5.65 ± 0.47	11.89 ± 0.60	3.80 ± 1.98	42.90 ± 4.14	76.00 ± 1.69
	63	6.10 ± 0.66	12.36 ± 0.70	4.95 ± 1.94	44.86 ± 6.02	73.50 ± 3.34
	70	6.25 ± 0.43	12.65 ± 0.75	5.53 ± 2.57	46.03 ± 1.86	73.88 ± 3.87
R50	49	6.20 ± 0.97	13.10 ± 1.95	2.95 ± 1.17	47.66 ± 6.81	77.25 ± 2.82
	56	5.02 ± 0.36	10.81 ± 0.57	3.58 ± 0.73	38.76 ± 3.21	77.25 ± 2.87
	63	5.46 ± 0.22	11.23 ± 0.65	3.78 ± 1.40	42.09 ± 2.00	77.13 ± 4.49
	70	5.67 ± 0.98	12.10 ± 0.69	5.16 ± 1.70	41.04 ± 9.46	74.13 ± 2.80
R65	49	5.70 ± 0.30	11.91 ± 0.67	3.60 ± 1.52	43.95 ± 1.69	77.25 ± 2.19
	56	5.06 ± 0.59	11.23 ± 0.65	4.71 ± 1.41	41.04 ± 4.66	79.63 ± 2.62
	63	5.54 ± 0.57	11.51 ± 0.80	4.70 ± 1.61	42.45 ± 3.24	77.00 ± 3.59
	70	5.76 ± 0.63	11.95 ± 0.66	5.34 ± 1.13	43.56 ± 4.81	75.88 ± 3.23
SEM		0.07	0.11	0.18	0.53	0.35
Significance						
Group		0.011	0.008	0.191	0.114	0.033
Age		< 0.001	< 0.001	< 0.001	0.013	0.002
Group × Age		0.218	0.095	0.911	0.392	0.248

AL - *ad libitum*; R50 - 50 g of feed per rabbit per day; R65 - 65 g of feed per rabbit per day
MCV - mean cell volume
SEM - standard error of the mean

erythrocyte counts increased again. The haemoglobin concentrations corresponded with erythrocyte counts and were significantly lower ($P \leq 0.008$) in both restricted groups. In addition, there was no effect of the feed restriction intensity on this characteristic. The haemoglobin concentration changed significantly with age ($P \leq 0.001$). The highest haemoglobin concentration was found at 49 days of age and the lowest at 56 days in all groups. Afterwards, the concentration steadily increased. The number of leukocytes ($P \leq 0.001$) and haematocrit ($P \leq 0.013$) significantly increased with advancing age, but was not affected by feeding regime. The MCV was significantly ($P \leq 0.033$) affected by feeding regime with higher values in restricted rabbits. However, at the end of restriction at 49 days of age, the restricted rabbits had lower MCV value than the ones fed *ad libitum*. In the following period, the MCV in all groups decreased ($P \leq 0.002$) with advancing age.

The biochemical blood characteristics of restricted rabbits and rabbits fed *ad libitum* are provided in Table 3. The protein metabolism is characterised by concentrations of total protein and urea. Concentrations of both indices were not affected by interaction of the feeding regime and age or by the feeding regime. Total protein concentration was significantly affected by age ($P \leq 0.001$). The highest total protein values were observed at 56 and at 70 days. Likewise,

Table 3. The effect of one-week intensive feed restriction on blood biochemical indices in broiler rabbits.

Group	Age (days)	Total protein (g/l)	Urea (mmol/l)	Glucose (mmol/l)	Cholesterol (mmol/l)	NEFA (g/l)	TAG (mmol/l)
AL	49	50.95 ± 6.45	7.88 ± 1.35	7.95 ± 2.01	2.37^{c} ± 1.06	0.61 ± 0.12	0.91 ± 0.36
	56	55.25 ± 6.48	7.59 ± 1.36	7.93 ± 1.27	3.69^{ab} ± 0.46	0.44 ± 0.13	0.60 ± 0.23
	63	47.51 ± 5.67	6.03 ± 0.82	7.29 ± 0.98	1.30^{d} ± 0.28	0.55 ± 0.19	1.58 ± 0.63
	70	54.71 ± 9.99	7.79 ± 1.02	8.71 ± 1.32	1.43^{d} ± 0.29	0.45 ± 0.19	1.34 ± 0.44
R50	49	44.46 ± 3.39	6.77 ± 0.77	7.63 ± 1.23	3.31^{b} ± 1.40	0.54 ± 0.15	0.46 ± 0.21
	56	50.23 ± 5.66	7.55 ± 1.37	7.79 ± 0.64	4.41^{a} ± 0.69	0.39 ± 0.07	0.48 ± 0.37
	63	44.88 ± 4.52	6.59 ± 1.61	8.03 ± 1.44	1.55^{d} ± 0.45	0.35 ± 0.03	1.23 ± 0.45
	70	59.42 ± 13.85	7.04 ± 1.04	7.09 ± 0.93	1.95^{cd} ± 0.63	0.39 ± 0.21	1.27 ± 0.49
R65	49	49.09 ± 4.52	8.09 ± 1.50	6.71 ± 1.13	4.24^{a} ± 0.90	0.45 ± 0.13	0.56 ± 0.33
	56	48.74 ± 7.24	7.43 ± 0.87	7.24 ± 1.48	4.22^{a} ± 0.79	0.48 ± 0.19	0.64 ± 0.38
	63	46.58 ± 5.83	6.90 ± 1.19	8.35 ± 2.01	1.56^{d} ± 0.41	0.37 ± 0.08	1.46 ± 0.56
	70	50.70 ± 4.24	7.46 ± 0.98	8.55 ± 1.24	1.30^{d} ± 0.49	0.28 ± 0.06	1.07 ± 0.24
SEM		0.80	0.13	0.14	0.14	0.02	0.06
Significance							
Group		0.158	0.252	0.582	0.123	0.003	0.048
Age		< 0.001	0.007	0.337	< 0.001	0.001	< 0.001
Group × Age		0.127	0.352	0.053	0.007	0.129	0.591

Different superscripts indicate significant differences (^{a,b,c,d} $P \leq 0.05$)
AL - *ad libitum*; R50 - 50 g of feed per rabbit per day; R65 - 65 g of feed per rabbit per day
NEFA - non-esterified fatty acid; TAG - triacylglycerides
SEM - standard error of the mean

the urea concentration was influenced only by age ($P \leq 0.007$) with similar trends to those of total protein. Lipid metabolism is represented by the concentration of cholesterol, NEFA and TAG. The significant interaction of the feeding regime and age ($P \leq 0.007$) was observed only in cholesterol concentration, when the highest values were found in group R50 at 56 days and in group R65 at 49 and 56 days of age. The feeding regime had a significant effect on NEFA ($P \leq 0.003$) and TAG ($P \leq 0.048$) concentrations. Lower concentrations were found in both restricted groups without an effect of the feed restriction intensity. The NEFA decreased significantly with age ($P \leq 0.001$), whereas TAG increased with age ($P \leq 0.001$).

Discussion

The results show that immediately after feed restriction, both restricted groups had lower live weights than AL rabbits which was not fully compensated because groups R50 and R65 reached 85.5% and 84.5%, respectively, of the final live weight of AL rabbits. These results agree with the data of Perrier (1998) who applied feed restriction with an intensity of 50 or 70% of AL for 3 weeks and detected a lower live weight in the restricted rabbits. Similarly in a study by Larzul et al. (2004) restricted rabbits of 8–18 weeks of age had lower live weights than the control group. Gidenne et al. (2009, 2012) also stated that feed restriction significantly decreased the final live weight depending on feed restriction intensity. On the other hand, Tůmová et al. (2007) did not find differences between restricted rabbits and rabbits fed *ad libitum* with the same period of feed restriction as in the present experiment, only their experiment finished at 84 days. The differing results could be caused by varying times of the beginning or duration of feed restriction. Rabbits with later feed restriction probably do not have sufficient realimentation period to fully compensate their live weights to similar to AL rabbits.

The values of all haematological characteristics studied in the present experiment were within the physiological range described by Hewitt et al. (1989) or by Archetti et al. (2008). Erythrocytes are involved in the transport of oxygen and carbon dioxide. Reduced erythrocyte counts in the restricted rabbits in our experiment did not correspond with the previous study of Tůmová et al. (2007) who did not detect an effect of feed restriction applied from 42 to 56 days of age in rabbits. On the other hand, Silas et al. (2014) observed lower erythrocyte counts in quantitatively restricted chickens compared to chickens fed *ad libitum*. This variability in erythrocyte counts of restricted rabbits and rabbits fed *ad libitum* may have been affected by different methods of feed restriction or its experimental timing or a different response of mammals and birds to feed restriction. The erythrocyte count values suggest that the restricted rabbits suffered from erythropoiesis which resulted in a lower erythrocyte concentration.

Haemoglobin is an iron-containing conjugated protein that performs the physiological function of transporting oxygen and carbon dioxide (Gbore and Akele 2010). In our study, haemoglobin changed with the feeding regime and age. On the other hand, Tůmová et al. (2007) did not find an effect of a two-week intensive feed restriction on the haemoglobin content in rabbits, and neither did Silas et al. (2014) in chickens. The decrease of the haemoglobin concentration in our study can be associated with the reduced erythrocyte counts in restricted rabbits.

Higher total leukocyte counts can be used for characterization of malnutrition as indicated in study of Melillo (2007). However, we did not detect an effect of the feeding regime on this haematological indicator in our study. These results were in agreement with Tůmová et al. (2007) who did not observe differences in leukocyte counts between rabbits fed *ad libitum* and restricted rabbits.

Haematocrit is a toxicity index. No differences of haematocrit values between the groups in our study agree with the results of Tůmová et al. (2007) in rabbits and with Silas et al. (2014) in chickens. Non-significantly lower haematocrit in restricted rabbits could have been related to lower growth rates and lower oxygen requirements of these groups as described by Boostani et al. (2010) in restricted chickens.

The MCV is the mean volume of erythrocytes. The one-week intensive feed restriction decreased the MCV at 49 days of age. In the realimentation period, both restricted groups had higher MCV than the AL group which is in contrast with the results of Tůmová et al. (2007) in rabbits or of Silas et al. (2014) in chickens who did not observe an effect of the feeding regime.

The biochemical blood characteristics are important for evaluation of the health status of animals and the level of their metabolism. The concentrations of total protein and urea are related to protein metabolism and were within the physiological range described by Archetti et al. (2008) and Özkan et al. (2012). Values were not influenced by the feeding regime which corresponds with Rebollar et al. (2011). The results indicate that the short intensive feed restriction did not affect synthesis and transfer of serum proteins and protein catabolism. It seems that the energy requirement for a growth of rabbits was presumably sufficient even in restricted rabbits because the protein and urea serum concentrations were not affected by the feeding regime which was in agreement with Rajman et al. (2006) and Van Harten and Cardoso (2010). Fat metabolism characteristics such as cholesterol, NEFA, and TAG concentrations were affected by the feeding regime. Cholesterol concentration of restricted rabbits increased at the end of the feed restriction in comparison with the AL rabbits which is not comparable with literature because of lack of data. On the other hand, during the realimentation period, differences between restricted and AL rabbits were not found which is in agreement with the results of El-Speiy et al. (2015) in rabbits and of Rajman et al. (2006), Boostani et al. (2010), and Silas et al. (2014) in chickens. The NEFA and TAG concentrations were lower in restricted rabbits than in AL rabbits after restriction and remained lower during the whole realimentation period. Similarly, van Harten and Cardoso (2010) observed lower NEFA and TAG concentrations at the end of their experiment with rabbits. Lower NEFA and TAG concentrations in the restricted rabbits in our experiment indicates lipid depletion of animals.

Based on our results, it can be concluded that feed restriction may affect erythrocyte counts, MCV, and haemoglobin concentration, and therefore, the transfer of oxygen. However, all measured values were within the physiological range for growing rabbits. No effect of the feeding regime on the protein metabolism was detected. However, cholesterol concentration may vary during restriction and in the realimentation period. The feeding regime significantly decreased NEFA and TAG concentrations which may indicate lipid depletion.

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