SERUM CHEMISTRY AND HEMATOLOGY VALUES IN COMMERCIAL RABBITS: PRELIMINARY DATA FROM INDUSTRIAL FARMS IN NORTHERN ITALY

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ABSTRACT

The aim of this preliminary study was to establish reference normal ranges of hematological and biochemical parameters in rabbits. A total of 8 industrial farms, located in Brescia, Milano and Bergamo Provinces (Northern Italy), were monitored during a 4-year period (2004-2007). Farms showed some variability as regards size, management and production parameters. Blood samples, collected from post weaning (30-45 days old) and growing (57-65 days old) rabbits, as well as restocking females before pregnancy (nulliparous), breeders at first pregnancy (primiparous), lactating does and breeders at culling (multiparous), were delivered within three hours to the laboratory.
The following analyses were performed on serum samples: alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, Phosphorus (P_i), creatine kinase (CK), lysozyme, lactate dehydrogenase (LDH) and creatinine. Blood samples were tested for: white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), platelets (PLT), hemoglobin (Hg), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean concentration of hemoglobin in the corpuscular volume (MCHC), mean corpuscular volume (MCV).
Results indicate that hematological and biochemical data are similar to those described in laboratory rabbits. In particular, the age-dependent trend of values was in agreement with previous observations. However, in does, a lower level of hemoglobin and lymphocyte numbers and a higher number of neutrophils and monocytes was observed, probably due to intensive production and exploitation. On the whole, this may indicate chronic stress under seemingly normal clinical conditions. LDH and CK values particularly differed from those described for laboratory rabbits. High urea and creatinine values in lactating does could be traced back to concurrent *E. cuniculi* infection.

Key words: Hematology, Serum chemistry, Rabbit, Welfare.

INTRODUCTION

Blood examination gives the opportunity to investigate the presence of several metabolites and other constituents and helps detect conditions of stress, which can be nutritional, environmental or physical (Aderemi, 2004). Physiological parameters (hormones, heart rate, immune reactions), when considered in relation with other parameters (behavior, morbidity), can be used as a welfare indicator (Hoy and Verga, 2007). Although there are a lot of studies on blood parameters of various domestic animals, few data are available about hematological values of farm rabbits, since little or no attention has been paid to rabbits reared for meat production. Consequently, we decided to investigate rabbits from industrial farms, where reproduction cycles are very fast and animals are intensively exploited.

The objective of this preliminary study was to collect a fairly high number of blood samples to establish reference ranges of hematological and biochemical parameters, referred to in previous studies (Yu *et al.*, 1979; Chiericato *et al*, 1985; Wolfoard *at al*, 1986; Hewitt *et al*, 1989; Amici *et al.*, 1998; Amici *et al*, 2000).

MATERIALS AND METHODS

Animals, farms and management

The study was performed between 2004 and 2007 in 8 industrial farms, located in the Po valley, in Brescia, Milano, and Bergamo Provinces (Northern Italy). Rabbit farms were closed-cycle operations, except one, and showed little variability as regards size, management and productivity. A total of 366 commercial hybrid rabbits were randomly selected from five production groups: post-weaning (30-45 days old) and growing (57-65 days old) rabbits, restocking females before pregnancy (nulliparous), breeders at their first pregnancy (primiparous), lactating does and breeders at the end of carrier (multiparous). The animals were clinically healthy and were given *ad libitum* access to commercial diet of about 16-17% protein and water provided by automatic nipples. The does were housed in individual cages, while post weaning and growing rabbits were group-housed in commercial cages with wire-mesh floors.

Collection of blood samples

Five-ml blood samples were collected from direct cardiac puncture in immobilized animals. This was done very quickly in order to limit the effect of acute stress on blood parameters. A 22-gauge sterile needle was used in all categories, except in 30-45 days old rabbits, where a 26-gauge needle was used. Half of the sample was expelled gradually into graduated tubes containing K3-EDTA (ethylenediamine tetracetic acid); then, tubes were immediately capped and mixed gently by repeated inversion. The rest of the sample was collected in sterile tubes without anticoagulant. All blood samples were transported to the laboratory at +4°C within 3 hours. Serum was separated and frozen at -20°C for biochemical analyses. Hematological analyses on EDTA samples were completed on the day of collection.

Hematological and biochemical studies

Blood samples with K3-EDTA anticoagulant were used for the determination of hematological parameters, i.e white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), blood hemoglobin (Hg), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These parameters were assessed by Cell-DYN 3500 Hematology Analyzer (Abbott Diagnostic Division, Santa Clara CA). Recommended settings and calibration for rabbit hematology were applied according to the manufacturer's operation manual.

Sera were used for determination of biochemical parameters, at 37 °C in a random-access clinical analyzer (SYNCRON CX5-DELTA, Beckman Coulter, Fullerton, U.S.A.) using kits by the same firm. The parameters and the respective methods applied are the following: aspartate aminotransferase (AST) - Henry method; creatine kinase (CK) - Rosalki method; alanine aminotransferase (ALT) - Henry method; lactate dehydrogenase (LDH) - pyruvate to lactate method; creatinine - colorimetric, Jaffè method; urea - enzymatic colorimetric, urease method; Inorganic phosphorus (P_i), - phoshomolybdate method. Serum lysozyme, a parameter of non-specific immunity, was investigated according to an established procedure (Amadori *et al*. 1997).

Statistical Analysis

Each set of data was analyzed using GraphPad Prism package, 2.1 Version (1999, GraphPad Software, Inc.).

RESULTS AND DISCUSSION

The number of animals analyzed for each category is shown in Tables 1, 2 and 3. Since the study is not yet finished and further samples are going to be taken and tested, the following results are to be considered preliminary. In particular the number of replicas from each farm is not sufficient to perform a statistical analysis in which the different units can be properly compared with each other. Therefore, at this point, we decided to simply perform a descriptive statistical analysis by grouping the animals according to age.

Hematological values are listed in Tables 1 and 2. Data from post-weaning rabbits showed that RBC count, concentration of hemoglobin and hematocrit were significantly lower ($P<0.05$) than in the other categories. Vice versa, the MCV was significantly higher. These results are in accordance with those of Bortolotti *et al.* (1989) in New Zealand laboratory rabbits. Interestingly, the hemoglobin values shown in Table 2 were always lower than those previously reported for all categories in laboratory rabbits (Bortolotti *et al.*, 1989; Moore, 2006).

Table 1: Values of hematological parameters (WBC) in each age group of rabbits

	Post-weaning rabbit		Growing rabbit		Nulliparous does		Primiparous does		Multiparous does	
White blood cells (10^9/l)	72[1]	5.4 ± 1.9 [2] 2.6 - 12.7 [3]	69	7.0 ± 2.1 3.3 - 12.2	58	7.2 ± 2.5 2.4 - 12.8	19	9.0 ± 3.9 3.9 - 18.4	83	8.7 ± 3.0 3.8 - 17.1
Neutrophils (%)	72	35 ± 13 10 - 66	69	51 ± 13 11 - 74	58	52 ± 12 21 - 73	19	52 ± 15 26 - 75	83	47 ± 14 15 - 77
Neutrophils (10^9/l)	72	1.9 ± 1.0 0.5 – 5.4	69	3.6 ± 1.5 0.7 - 8.5	58	3.7 ± 1.5 1.1 - 7.4	19	4.5 ± 2.3 1.9 - 11.6	83	4.1 ± 2.0 1.3 - 10.3
Lymphocytes (%)	72	47 ± 16 20 - 79	69	29 ± 15 7 - 70	58	29 ± 14 9 - 64	19	30 ± 17 10 - 62	83	34 ± 15 7 - 78
Lymphocytes (10^9/l)	72	2.5 ± 1.1 0.8 - 6.4	69	2.1 ± 1.4 0.5 - 6.4	58	2.1 ± 1.4 0.5 - 6.5	19	3.0 ± 2.6 0.7 - 8.6	83	3.0 ± 2.0 0.4 - 10.2
Monocytes (%)	72	13 ± 6 0.5 - 28	69	15 ± 5 4 - 29	58	15 ± 5 1 - 32	19	14 ± 7 5 - 34	83	15 ± 5 3 - 33
Monocytes (10^9/l)	72	0.7 ± 0.5 0.0 - 3.4	69	1.0 ± 0.4 0.1 - 2.4	58	1.1 ± 0.6 0.0 - 3.7	19	1.3 ± 0.9 0.3 - 4.2	83	1.3 ± 0.7 0.1 - 5.0
Eosinophils (%)	72	0 ± 0.1 0.0 - 0.5	69	0 ± 0.1 0.0 - 0.5	58	0 ± 0.1 0.0 - 0.7	19	0.1 ± .0 0 - 0.3	83	0.0 ± 0.3 0 - 2
Eosinophils (10^9/l)	72	0.01±0.01 0.00-0.03	69	0.01±0.01 0.00-0.04	58	0.01±0.01 0.00-0.04	19	0.01±0.01 0.00-0.03	83	0.01±0.01 0.00-0.14
Basophils (%)	72	3 ± 2 0 - 13	69	3 ± 2 0.5 - 9	58	2 ± 1 0 - 7	19	2 ± 1 0 - 6	83	2.3 ± 1.5 0 - 6
Basophils (10^9/l)	72	0.1 ± 0.1 0.0 - 0.6	69	0.2 ± 0.1 0.0 - 0.7	58	0.1 ± 0.1 0.0 - 0.4	19	0.1 ± 0.1 0.0 - 0.4	83	0.1 ± 0.1 0.0 - 1.0

[1]Number of animals; [2]Mean and standard deviation; [3]Minimum to maximum range

Table 2: Values of hematological parameters (RBC-PLT) in each age group of rabbits

	Post-weaning rabbit		Growing rabbit		Nulliparous does		Primiparous does		Multiparous does	
Blood Red cells (10^{12}/l)	73[1]	4.7 ± 0.4[2] 3.5 - 6.6[3]	69	5.5 ± 0.3 4.6 - 6.6	58	5.7 ± 0.5 3.9 - 7.0	20	5.4 ± 0.4 4.7 – 6.4	83	5.3 ± 0.6 3.4 - 6.8
Hemoglobin (g/dl)	73	10.4 ± 0.8 6.7 - 12.7	69	11.5 ± 0.8 9.5 - 13.7	58	12.3 ± 1.0 7.8 - 15.4	20	11.6 ± 1.0 9.3 – 13.6	84	11.1 ± 1.3 7.3 - 14.3
Hematocrit (%)	73	28.8 ± 2.3 18.9 - 34.7	69	31.1 ± 2.2 25.5 - 37	58	32.1 ± 2.7 20.6 - 39.6	20	30.7 ± 2.5 25.6 - 36.0	84	29.5 ± 3.2 19.5 - 36.6
MCV (fl)	73	60 ± 3 49 - 66	69	56 ± 1 52 - 60	58	55 ± 2 50 - 61	20	56± 2 52 - 61	84	55 ± 2 50 - 61
MCH (pg)	73	21 ± 1 17 - 23	69	20 ± 0.7 19 - 23	58	21 ± 1 18 - 23	20	21 ± 1 19 – 23	84	20 ± 1 14 - 23
MCHC (g/dl)	73	36 ± 1 33 - 43	69	36 ± 0.7 35 - 38	58	38 ± 0.7 36 - 39	20	37 ± 0.6 36 – 39	84	37 ± 1 26 - 40
Platelets (10^9/l)	73	427 ± 135 75 - 763	69	431 ± 98 201 - 716	58	400 ± 89 192 - 662	20	425 ± 116 275 – 616	84	393 ± 131 75 - 866

[1]Number of animals; [2]Mean and standard deviation; [3]Minimum to maximum range

Biochemical values are listed in Table 3. The analysis of such values is hampered by the lack of reference data for different age groups. Nevertheless, we tried to correlate our findings with the age of rabbits. A higher degree of homology for most parameters was found in post weaning and growing rabbits, whereas significant differences were detected between nulliparous does and lactating, multiparous does.

Table 3: Biochemical parameters in each age group of rabbits

Parameter	Post-weaning rabbit		Growing rabbit		Nulliparous does		Primiparous does		Multiparous does	
Lysozime (µl/ml)	69[1]	10 ± 7[2] 1 – 34[3]	82	12 ± 6 1 - 39	91	9 ± 10 0.4 - 74	23	15 ± 9 1 - 34	76	16 ± 8 1 - 39
AST (IU/l)	72	51 ± 24 18 - 37	86	45 ± 16 17 - 98	99	42 ± 22 12 - 138	24	61 ± 31 22 - 164	75	37 ± 25 14 - 105
ALT (IU/l)	73	31 ± 9 14 - 3	91	38 ±11 19 - 73	100	42 ± 11 17 - 81	25	38 ± 11 16 - 71	75	33 ± 12 12-81
CK (IU/l)	72	2035 ± 854 492 - 4354	88	2042 ± 941 655 - 5583	100	1314 ± 799 1263 - 2954	22	1540 ± 664 631 - 3342	73	1042 ± 703 286 - 3534
LDH (IU/l)	61	1431 ± 626 371 - 3679	69	1525 ± 552 559 - 3497	99	867 ± 438 1321 - 1808	23	1398 ± 545 591 - 2927	73	860 ± 580 187 - 2615
Urea (mmol/l)	10	5.2 ± 0.8 3.6 - 6.6	20	5.0 ± 0,7 4.2 - 6.8	99	7.0 ± 1.4 4.7 – 11.9	4	9.8 ± 1.0 8.3 - 10.8	56	8,1 ± 1,7 4.3 – 13.5
P_i (mmol/l)	10	2.86 ± 0.23 2.51 - 3.19	20	2.21 ± 0.17 1.93 - 2.48	100	1.68 ± 0.38 0.94 - 2.41	5	0.98 ± 0.23 0.78 – 1.38	56	1.45 ± 0.35 0.90 – 2.17
Creatinine (µmol/l)	10	53 ± 7 37 - 65	20	64 ± 17 53 - 89	99	82 ± 19 47 - 147	4	90 ± 7 84 - 101	57	92 ± 13 68 – 131
Proteins (g/l)	72	42 ± 5 32 - 58	90	48 ± 4 32 - 61	100	55 ± 6 36 - 73	25	46 ± 11 22 - 67	77	55 ± 8 21 - 69

[1]Number of animals; [2]Mean and standard deviation; [3]Minimum to maximum range

Lysozyme was basically increasing with age and there was a large intersubject variability. AST e ALT values overlapped those reported by other authors in laboratory rabbits (Chiericato *et al*., 1985; Wolford *et al*., 1986). CK and LDH values were particularly high even if compared with other studies based on cardiac intrapuncture (Chiericato *et al*., 1985; Wolford *et al*., 1986). As opposed to other species (i.e. turkey, personal observations) the LDH and CK values were higher in post weaning and growing rabbits than in adult breeders. Urea and creatinine were higher in does than in young rabbits. High blood concentrations of urea and creatinine are usually associated with renal disease and antibody response to *Encephalitozoon cuniculi* (Harcourt-Brown, 2007). Serum phosphate was higher in post-weaning rabbits and decreased in other categories. According to Rosol and Capen (1997), it may be due to growth hormone-driven phosphate renal reabsorption. The total protein values were similar to those previously detected in other types of rabbits; protein was also shown to increase with age as in many other species (Kaneko *et al.*, 1997).

CONCLUSIONS

This study reports preliminary data that could be partly modified with a larger sample. Further investigations will certainly enable us to perform a more complete and detailed statistical analysis. Having said that, this study could show interesting differences in terms of hematological and biochemical parameters between laboratory and commercial farm rabbits. In particular, we found significant differences according to age/category of rabbits.

The sampled rabbits were randomly chosen and all of them were apparently healthy. However, some of them were possibly suffering from distress and/or sub-clinical, chronical infections when sampled. The width of the range between minimum and maximum values for several parameters could be due to inter-subject variability occurring in non-healthy animals. On the other hand, the standard deviation was generally narrow indicating that these differences in hematological and biochemical values could be caused by nutritional, environmental and hormonal factors typical of each industrial farm. Therefore, a specific analysis and comparison between different units has to be planned and

performed. Further investigations are needed to better understand the usefulness of clinical chemistry and hematology for assessing the health and welfare of rabbits in commercial farms.

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