PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

Session
Nutrition & Digestive Physiology

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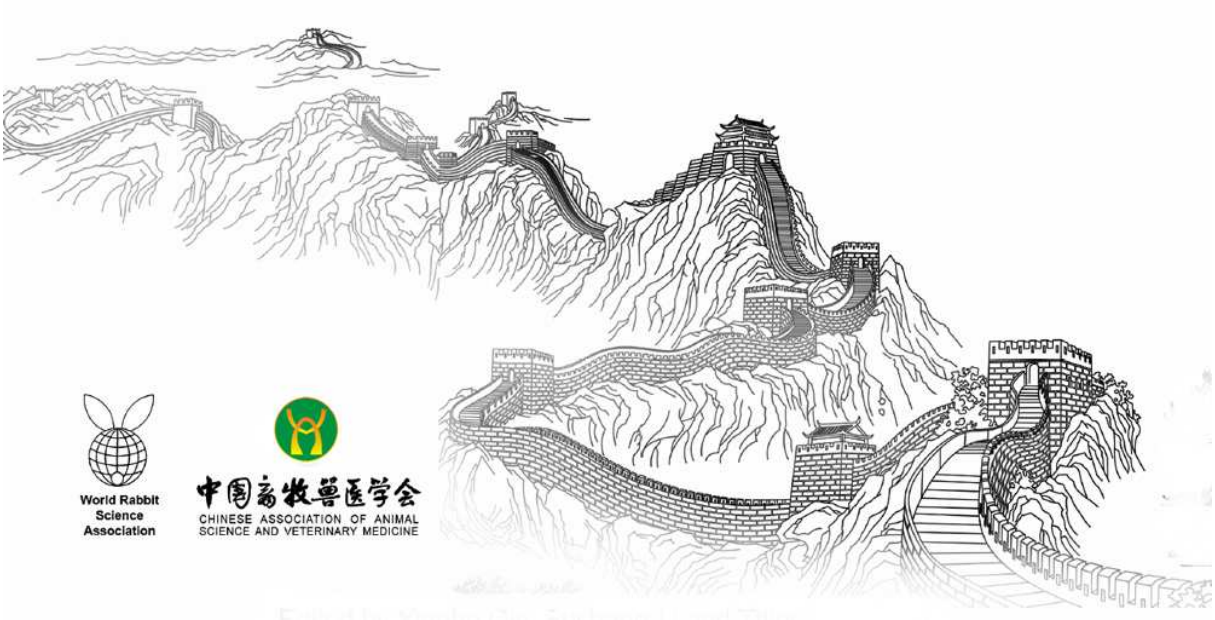
Full text of the communication

How to cite this paper :
Bălăceanu R.A., Cotor G., Codreanu I., Stoica L., Dojană N., 2016 - Changes in blood parameters in post-weaning rabbits fed by different fibre or starch dietary level. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 261-264.*



CHANGES IN BLOOD PARAMETERS IN POST-WEANING RABBITS FED BY DIFFERENT FIBRE OR STARCH DIETARY LEVEL

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ABSTRACT

The objective of this study was to evaluate the effect of different fibre or starch dietary levels on the blood parameters in post-weaning rabbits. A control group was fed a post-weaning growing rabbit diet containing 138 g/kg dry matter (DM) crude fibre and 168 g/kg DM starch. Three rabbit groups were fed diets having either 147, 156 or 164 g/kg DM crude fibre level, and three groups were fed diets having 190, 223 or 247 g/kg DM starch level. The experiment started with 6 weeks old rabbits and lasted 28 days. Blood glucose values were on average 7% lower in the groups fed by fibre-enriched diets vs. control (P = 0.048) and on average 10% higher in the rabbit groups fed by starch-enriched diets vs. control. Higher differences were found in rabbit groups fed on higher levels of fibre or starch. Total protein level was not significantly modified, excepting a 13% increase in fibre-diet fed groups and a 13% in starch-diet fed groups of the globulin fraction and a subsequent decrease (8.3% and, respectively 13.8%) of the albumin/globulin ratio, but no differences according to the level of fibre or starch. Compared to control, blood urea was 17% lower in high crude fiber diet, and was 10% higher for the high starch diet fed. Alanin aminotransferase, aspartate aminotransferase and alkaline phosphatase showed a relative steady-state activity. Positive relationship of fibre diet variable with protein (r = 0.87) and LDH cholesterol (r = 0.78), and negative with glucose (r = 0.72), urea (r = 0.91) and triglycerides (r = 0.78) were found. Dietary starch level correlated positively with triglycerides (r = 0.92) and LDL cholesterol (r = 0.86). Negligible relationship was found between cholesterol and fibre (r = 0.15) or starch (r = 0.01) enriched diet variable.

Key words: blood biochemichemistry, fibre, starch, growing rabbit.

INTRODUCTION

In rabbit physiology research as well as in rabbit metabolism or nutrition researches, diets differing by structure or chemical composition are tested. Most diets experiment the effect of different levels of protein, starch or fibre (Eiben *et al*., 2008, El-Tahan *et al*., 2012, Wang *et al*., 2012). Structure and composition of these diets, sometimes very different from those of standard diet, could influence blood parameters and, subsequently, the physiological status and welfare also (Atansuyi *et al*., 2012, Ewuola *et al.*, 2012). The aim of this research was to reveal the extent to which different starch or fibre dietary levels could influence hematological parameters of the growing rabbits.

MATERIALS AND METHODS

Animals and experimental design

Research was performed on 6-week old New Zealand x Californian (males and females) weaned rabbits of an initial weight of 1,038 ± 54 g. Kits were weaned at 28 days of age. Two weeks later, the young rabbits were equally divided in seven groups: a control and six experimental groups (10 rabbits each one). Rabbits were housed in the same room, where the temperature ranged between 22°C and 26°C, in individual cages (250mm × 400 mm × 300 mm). Water and pelleted feeds were provided *ad libitum*. A cycle of 12 h from 7:00 to 19:00 of light was used throughout the trial. Diets were

calculated according to weaned rabbit requirements (De Blas and Mateos, 2010): a control diet, three diets with increasing crude fibre level (FL1, FL2, and FL3) and three diets with increasing starch level (SL1, SL2, SL3) (Table 1). All diets were made based on maize, barley, oat, wheat bran, full-fat soya, sunflower seed meal, dehydrated alfalfa meal and a vitamin mineral premix (providing per kg diet : 10,000 IU vitamin A, 2,500 IU vitamin D_3, 80 IU vitamin E acetate, 2.0 mg thiamine, 4.5 mg riboflavin, 3.4 mg pyridoxine, 10 mg pantothenic acid, 20 mg niacin, 0.030 mg vitamin B_{12}, 100 mg Fe, 100 mg Zn, 52.2 mg Mn, 0.4 mg I and 0.4 mg Se. Fibre enrichment was done by adding wheat straw and bran in partial substitution of maize and barley, and starch-enriching was done by adding maize starch in partial substitution of maize and dehydrated alfalfa meal: FL1 = 6.0 % fibre enriched diet, FL2 = 12.7% and FL3 = 18.8% ; SL1 = 13.7% starch enriched diet, SL2 = 33.4%, SL3 = 47.4%. The trial lasted 28 days from 42d to 70d old. Feed intake and body weight were monitored and the animals were also clinically supervised.

Table 1. Calculated chemical composition of control and experimental post-weaning rabbit group diets

Chemical composition	Control	Fibre enriched diets			Starch enriched diets		
		FL1	FL2	FL3	SL1	SL2	SL3
Dry matter (DM, g/kg)	886	883	882	878	885	892	889
Crude protein (g/kg DM)	127	138	136	135	128	127	119
Crude fibre (g/kg DM)	138	147	156	164	121	123	121
Starch (g/kg DM)	167	149	147	146	190	223	247
Digestible energy (MJ/kg DM)*	10.04	9.93	9.51	9.28	99.34	10.72	10.73

* Calculated according to energetic values of ingredients (tables INRA, 1999)

Blood sampling and chemical analyses

At the end of the experimental feeding period, blood was sampled from marginal ear vein, at 10:00 h, using heparin as anticoagulant. Blood was sampled randomly from 7-8 rabbits from control and from each experimental group. Fresh blood samples were used to analyze the blood morphological parameters: red blood cells (RBC), hemoglobin, packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) using a ABACUS Junior Vet automatic hematology analyzer. The blood serum was obtained after blood coagulation, centrifugation 10 min at 14,000 G and decantation. The sera were stored at 4°C until biochemical processing, but no more than 24 hours. Glucose was measured by an enzymatic method (Ashwell, 1957). Total serum protein and serum albumin were determined according to Gornall *et al*. (1949). Alkaline phosphatase (ALP, EC 3.1.3.1), aspartate aminotransferase (AST, EC 2.6.1.1) and alanin aminotransferase (ALT, EC 2.6.1.2) activities were assayed by spectrophotometric methods according to Richterich (1967). Triglycerides, total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and urea were measured according to Manta *et al*. (1976).

Statistical analysis

Descriptive statistics and statistical comparison of blood parameters of rabbit groups were performed by two-way ANOVA, using a General Linear Model procedure of SAS (2002). When any null hypothesis was rejected Tukey's post hoc test was performed to compare the groups. The relationship between starch or fibre enrichment level variable and the blood biochemical variables was statistically analyzed by Person correlation coefficient (r). The significance level of null hypothesis rejection was stated for $P < 0.05$.

RESULTS AND DISCUSSION

Results of intake and growth (table 2) suggested that animals kept their physiological status regardless the fibre or starch dietary level. A 14.7% starch supplementation (in SL1) resulted in a higher intake and growth: +25% and +63%, respectively.

The dietary fibre level enriched diets as well as the starch level didn’t modify the blood morphological parameters (P>0.05, table 3). Surprisingly, RBC seems correlated with starch and fibre level. Keeping

in physiological limits of these blood morphological features shows that hematopoietic process was held still in the normal range, at least during the experimental period and for the tested levels of fibre or starch.

Table 2. Daily gain and feed intake of rabbit fed on increasing fibre or starch dietary level.

Item	Control (n=10)	FL1 (n=10)	FL2 (n=10)	FL3 (n=10)	SL1 (n=10)	SL2 (n=10)	SL3 (n=10)	P values
Daily gain (g/d)£	21.1	28.4	22.4	21.4	34.3*	30.4	20.6	0.005
Feed intake (g/d)	81.5	87.0	79.0	75.5	101.6*	98.8*	94.6	0.019

n= number of animals; *: significant difference from the control group at the level P < 0.05. £: period = 42 to 70d old.

Table 3. Morpho-hematological parameters in post-weaning rabbits fed for 28 days on increasing fibre or starch dietary level

Item	Con-trol	Fibre enriched diets				*P* values	r	Starch enriched diets				*P* values	r
		FL1	FL2	FL3	SD			SL1	SL2	SL3	SD		
n=	8	8	7	8				10	9	9			
RBC (10^6/µL)	6.58	6.65	6.50	6.36	0.4	0.059	0.81	6.50	6.44	5.89	0.4	0.054	0.86
Hemoglobin (g/dL)	10.6	11.0	10.7	9.8	1.5	0.059	0.66	10.0	10.5	10.2	1.0	0.932	0.22
PCV (%)	36.7	37.7	36.4	35.0	2.3	0.069	0.71	36.4	36.8	36.0	3.0	0.060	0.56
MCHC (g/dL of RBC)	28.8	29.1	29.3	28.0	1.3	0.069	-	27.4	28.6	28.3	3.3	0.491	-
MCV (fL)	55.7	56.6	56.0	55.8	3.0	0.088	-	56.0	57.1	61.1	2.9	0.059	-
MCH (pg)	16.1	16.5	16.4	15.4	0.5	0.831	-	15.3	16.3	17.3	2.5	0.048	-

SD: standard deviation; n = sample size; r = correlation coefficient; RBC = red blood cells; PCV = packet cell volume MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin

Regarding the effects of both, fibre enriched diets and starch enriched diets on the blood biochemical parameters, the effects were quite different. Blood glucose lowered 3% in FL1, 12% in FL2 and 7% in FL3 (vs. control, Table 4) and increased by 12% in SL1, 7% in SL2 and 11% in SL3 (vs. control). It is worth noting that in the case of both, fed by fibre enriched diet or fed by starch enriched diet groups, *P* values were very close to 0.05. Keeping in normal limits of the blood glucose concentration shows the ability of the rabbits to control the intermediary metabolism of carbohydrates for the tested fibre or starch dietary levels.

Table 4. Blood biochemical parameters in post-weaning rabbits fed for 28 days on increasing fibre or starch dietary level.

Item	Control	Fibre enriched diets			SD	*P* values	r	Starch enriched diets			SD	*P* value	r
		FL1	FL2	FL3				SL1	SL2	SL3			
n	*10*	*10*	*10*	*10*				*10*	*10*	*10*			
Glucose*	$87.0^{a;b}$	84.6	77.0^{a}	81.4^{b}	2.2	0.048	0.72	99.0	94.0	97.5	6.60	0.055	0.59
Protein**	7.3	7.3	7.5	7.5	4.4	0.509	0.87	7.7	7.5	7.6	0.40	0.090	0.47
Albumin (A)**	3.7	3.7	3.5	3.6	0.6	0.454	0.65	3.7	3.3	3.6	0.33	0.193	0.52
Globulins (G)**	3.4	3.6	4.0	3.9	0.8	1.054	0.88	3.9	3.9	3.8	0.55	0.232	0.62
A/G ratio	1.08	1.02	0.87	0.92	-	-	0.85	0.94	0.85	0.94	-	-	0.69
Urea (mmol/L)	$6.9^{a;b;c}$	6.7	5.5^{a}	5.5^{b}	0.4	0.031	0.91	5.2^{a}	5.2^{b}	5.8^{c}	0.23	0.002	0.51
AST***	50.3	55.4	52.3	49.4	3.0	0.065	0.24	50.0	52.9	46.4	5.0	0.094	0.38
ALT***	38.8	36.9	40.1	42.0	1.7	0.298	0.63	35.9	38.4	33.0	3.7	0.059	0.67
ALP***	104	120	110	96.0	6.4	0.243	0.39	120	110	114	8.6	0.165	0.34
Cholesterol*	89.9	87.7	92.3	89.3	8.8	0.404	0.15	85.0	79.0	92.1	4.4	0.332	0.01
Triglycerides*	$60^{a;b}$	48^{a}	46	48	2.7	0.020	0.78	55^{b}	54	44	1,7	0.044	0.92
HDL chol.*	$35^{a;b}$	29	32^{a}	30	2.4	0.039	0.61	29	33^{b}	31	2.0	0.047	0.33
LDL chol.*	$40^{a;b}$	54	50^{a}	54	6.2	0.033	0.78	46^{b}	46	48	2.3	0.022	0.86

Legend: as in Table 2. Means with the same letter in the same row did not differ (P<0.05); * mg/dL; ** g/dL; *** IU ;

Total protein level was not modified, except a little increase of the globulin fraction (+13%, in FL1-FL3 and 14% in SL1-SL3 groups), and a subsequent decrease (8% and 14%) of the albumin/globulin ratio. Positive correlation of protein and globulins with fibre level were found (r = 87 for protein and r = 0.88 for globulins). These results disagree with Oboh *et al*. (2007), whose showed that the total protein, albumin and globulin were lowered (*P*<0.05) in rabbits after 8 weeks of a high carbohydrate/low fat diet.

Blood urea was lower in all the experimental groups vs. control, regardless the levels of fibre or starch in the experimental diets. The differences became significant in FL2 group and in SL1 group. Urea level decreased already in FL1 (-3%), and further decreased in FL2 and FL3: 22% ($P<0.05$). Urea was also reduced in SL groups: 32% SL1 and SL2 and 19% in SL3 ($P<0.05$). The levels of urea correlated negatively with the fibre level ($r = -0.91$), and weakly negative with starch level ($r = -0.51$). The decrease of urea level suggested a decrease of protein mobilization and consequently a decrease of protein use as a metabolic fuel.

The almost steady-state activities of ALT, AST and ALP could be interpreted as integrity of liver health parameters and maintaining a constant protein synthesis activity of the liver regardless the starch or fiber dietary level.

Increasing the fibre or starch level did not change total cholesterol ($P > 0.05$) but affected the lipid metabolism profile. No relationship were found between cholesterol and fibre level ($r = 0.15$) or starch ($r = 0.01$). Triglycerides and HDL was lower in FL1 (-20% and -17%, resp.) and in SL1 (-8% and -17%, resp.). A strong negative relationship of triglycerides with starch diets was found ($r = -0.92$). On the other hand, LDL increased in FL1 and in SL1 compare to control. The level variations of the triglycerides, HDL or LDL were not necessarily proportional to the levels of fibre or starch which could relieve a high physiological capacity of the rabbit to correct the levels of these blood parameters.

CONCLUSION

Increasing fibre or starch dietary levels affected the blood biochemical parameters of the weaned rabbits. Blood glucose and protein levels were slightly modified. Urea correlates with the fibre and starch dietary level. Triglycerides and HDL decrease in both, fibre and starch enriched diets. The level variations of the triglycerides, HDL or LDL were not proportional to the high fibre or starch dietary level.

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