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PROTEINS AND FATS IN THE SERUM OF RABBITS FED DIFFERENT QUANTITIES OF DRIED OLIVE CAKE

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Abstract

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The subject of this research is the nutritive effect that different quantities (10 and 20%) of dried olive cake had on total proteins and the proportion of individual proteins, on triacylglycerols, on total cholesterol, HDL and glucose in the serum of fattened rabbits following a 56-day experiment. The experiment involved 60 rabbits, divided into three groups: a control group (C) and two test groups (E_1 and E_2), each comprising 10 males and 10 females. Throughout the experiment, rabbits in group C received no olive cake in their feed; group E_1 rabbits were fed a feed mixture containing 10% of dried olive cake; group E_2 rabbits received a feed mixture containing 20% of dried olive cake. After the 56th day of the experiment, blood samples were obtained by cardiac puncture, and specific haematological variables, total proteins and their fractions, triglycerides, total cholesterol, HDL and glucose were established in the serum.

The results obtained show no significant differences in the concentration of total proteins, albumin, alpha$_1$, alpha$_2$, beta and gamma globulin in the serum of rabbits in control group and in the two test groups. Similarly, no significant differences were found in the concentration of serum triacylglycerols and glucose between group C rabbits and the two test groups.

However, group E_1 rabbits did show a significantly lower concentration of cholesterol compared with those in group E_2; group E_1 rabbits also had a significantly ($P<0.01$) lower concentration of HDL in their serum than those in group E_2. Results of the study show that 10% and 20% of dried olive cake had neither detrimental effect on health, nor did they cause significant changes in the level of proteins, lipids and glucose in the serum of rabbits after a 56-day feeding period. Addition of dried olive cake into rabbit diets may therefore be recommended.

Hyla rabbits, feeding, olive cake, serum, proteins, lipids and glucose

Cultivation of olives has a long-standing tradition in many countries throughout the world, but it is particularly well developed in the Mediterranean area. Olive cake is the industrial by-product left following the extraction of oil from olives. The resulting considerable quantities of olive cake can be used as food for certain categories of livestock. Alternatively, they are simply deposited in the area around oil extraction plants where, due to their long period of degradation, they pollute the environment for prolonged periods of time. For certain types of animals (ruminants, rabbits, etc.) olive cake can be used as feed in somewhat greater quantities, while for chickens and pigs olive cake should form only a smaller portion of their daily diet. Depending on the technological procedure used in the extraction of olive oil, the resulting olive cake contains 3.5 - 8.5% of oil, 2.5 -6.0% of raw proteins and other nutrients (Manoukas et al.; 1973, Rupić et al.; 1993, Vešnik et al.; 1993, 1994).

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Olive cake also contains a high volume of raw fibre (220-350 g· kg^{-1}) which can be harmful when used in the diet for poultry and pigs but is beneficial to ruminants and rabbits. When obtained by using the centrifugal separation process (Van Soest 1975; Martilotti 1983; Sansoucy 1985) olive cake retains a high content of water (430-570 g·kg^{-1}) and it is therefore necessary for it to be allowed to drain well before it is fed. When added to fodder mixtures, however, it should be completely dried.

Available literature offers only limited data related to the use of olive cake as feed for rabbits, in particular for fattened rabbits. Within the number of our research projects focused on the use of olive cake as feed for animals, we have also conducted this experiment with fattened rabbits, the aim of the experiment being to establish possibilities for the addition of olive cake to their diet.

The aim of the present study was to establish the nutritive effect of larger quantities (10 and 20%) of dried olive cake, that is to say, of its chemical composition, on the health of rabbits, based on specific biochemical haematological indicators, and by defining the quantities of total serum proteins and the proportion of individual proteins, triacylglycerols, total cholesterol, HDL and glucose following a 56-day trial feeding period.

Materials and Methods

Animals and diets

Sixty (30 male + 30 female) 30-day-old weaned Hylla rabbits were used. The rabbits were divided into 3 groups comprising 20 animals each: control group (C) and two experimental groups (E_1 and E_2). All animals were tattooed for identification. Initial average body mass of group C rabbits was 666.15 ± 78.99 g, while in experimental groups it was 659.93 ± 83.42 g (E_1) and 648.25 ± 82.27 g (E_2). Analysis of variance showed that differences in the initial body mass of rabbits assigned to the different groups were not significant. Each group of rabbits (10 males + 10 females) was housed in five stainless steel cages, located in the same room and on the top of each battery. The room was air-conditioned; the temperature was maintained between 18 and 20 °C, with a relative humidity of between 60 and 70 %.

The basic chemical composition of olive cake is shown in Table 1.

Table 1
Chemical composition of olive cake (g·kg^{-1}) after oil extraction

	Basic chemical composition							
	Moisture	Ash	Crude protein	Crude fat	Crude Fibre	N F E*	Ca	P
Fresh olive cake	487.2	8.1	27.9	39.2	259.8	177.8	0.7	0.4
Dried olive cake	19.5	15.6	53.4	75.0	497.0	339.5	1.4	0.8

NFE* = nitrogen-free extract

Throughout the whole period of the experiment, group C rabbits received a diet containing no olive cake, while those in group E_1 received fodder containing 10%, and group E_2 20%, dried olive cake. The composition of fodder mixtures and their chemical analyses (A.O.A.C., 1984) is shown in Table 2.

Table 3 presents the amino acids composition of fodder mixtures (Rhône Poulenc Animal Nutrition 1989) and of dried olive cake (A.O.A.C. 1984).

Throughout the entire 56-day experimental period the rabbits were fed the previously described mixtures (Table 2). Olive cake used in the experiment was obtained through the centrifugal separation process (Martilotti 1983), in which oil was extracted from local varieties of olive. Fresh olive cake, containing 487.2 g·kg^{-1} water, was dried at a temperature of 35 °C to a water content of 19.5 g·kg^{-1} and was then reduced to 2-mm-size particles in a hammer mill. All rabbits involved in the experiment were fed from automatic feeders. Water was provided *ad libitum* from automatic dispensers.

Microbiological examination of one gram of fodder mix showed a germ count of 270,000 for group C; 22,000 for group E_1 and 49,000 for group E_2. In the same quantity (1 g) of fodder, no salmonellae were found, while

Table 2
Ingredients and chemical composition of diets

Ingredients (% as fed)		Groups of animals		
		C (control)	E_1	E_2
Dried olive cake		0.00	10.00	20.00
Corn		28.40	31.90	34.70
Barley meal		5.00	3.00	3.00
Wheat middlings		6.00	5.00	6.00
Soyabean meal		0	10.00	17.40
Sunflower meal		18.50	11.00	8.00
Dehydrated alfalfa meal		41.00	27.80	9.00
Dibasic calcium phosphate		0	0	0.20
Limestone		0.30	0.50	0.90
Sodium chloride		0.30	0.30	0.30
Mineral-vitamin mix		0.50	0.50	0.50
Total		100.00	100.00	100.00
Analysis as fed, **g in dry matter***				
Dry matter	$g \cdot kg^{-1}$	881.00	888.10	887.60
Crude protein	$g \cdot kg^{-1}$	**224.70**	**226.80**	**224.20**
Crude fat	$g \cdot kg^{-1}$	**42.50**	**43.90**	**52.95**
Crude fibre	$g \cdot kg^{-1}$	**197.20**	**202.50**	**189.50**
Ash	$g \cdot kg^{-1}$	**67.65**	**63.10**	**53.00**
NFE[&]	$g \cdot kg^{-1}$	**348.95**	**351.80**	**367.95**
Ca	$g \cdot kg^{-1}$	10.44	9.35	8.56
P	$g \cdot kg^{-1}$	7.38	6.19	5.97
Metabolisable Energy	MJ/kg**	12.86	12.71	12.62

***A. O. A. C. methods used troughout (1984)**
[&]**NFE = nitrogen-free extract**
**Calculated data (Allen 1993)

Table 3
Amino acid composition of fodder mixes* and dried olive cake** used in experiment ($g \cdot kg^{-1}$ in mixtures and dried olive cake)

Amino acids	C (control)	E_1	E_2	Dried olive cake
Methionine	3.30	3.10	3.00	0.20
Lysine	6.70	7.80	8.50	0.70
Threonine	6.90	7.00	6.80	2.80
Arginine	10.80	11.10	11.80	2.40
Glycine	9.00	8.20	7.60	3.10
Histidine	4.20	4.30	4.30	0.70
Phenylalanine	8.90	8.70	8.50	2.70
Leucine	13.60	13.80	13.60	4.20
Isoleucine	8.00	8.00	7.70	2.30
Valine	8.60	11.00	14.90	3.30
Serine	6.90	10.40	13.60	2.90
Tyrosine	5.10	6.50	7.90	0.60

* Calculated data (Rhône Poulenc Animal Nutrition 1993)
** Amino acids composition by analysis

sulphite-reducing clostridia did not exceed 1 000 in none of the mixtures used. One gram of control mixture showed a mould count (*Aspergillus* sp., *Penicillium* sp.) of 4 000. In group E_1 the count was less than 1 000, while in group E_2, test fodder mixture showed a mould count of 2 000.

Blood samples for biochemical and haematological analyses were obtained at the end of the experiment by cardiac puncture. Prior to puncture the rabbits were narcotised with an intramuscular injection of Ketamine

hydrochloride, 50 mg/kg body mass (Makek et al. 1981). The blood was stored in Greiner test tubes. Blood samples (5 ml) were taken from all 60 rabbits involved in the experiment. Variables determined in serum were: concentration of total proteins, level of albumin, alpha$_1$, alpha$_2$, beta and gamma globulin, concentration of triacylglycerols, total cholesterol, HDL and glucose. Serum triacylglycerols, cholesterol, HDL and glucose in serum were determined by applying Olympus reagents using Olympus AU-600 (1997) apparatus. Total plasma proteins were determined the Wolfson et al. method (1948), while individual fractions of the serum proteins were determined by electrophoresis. Electrophoretic separation of serum proteins was done on cellogel strips, and readings of individual serum protein fractions were carried out with a densitometer, produced by Biotecnica Instruments BT-512.

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards as laid down by the Croatian Ministry of Agriculture.

Statistics

Statistical analyses were performed using the GLM and UNIVARIATE procedure of the SAS (1989).

Results

After 56 days of feeding, the following mean body masses were achieved: 2334.50 ± 186.30 g (C), 2341.00 ± 222.06 g (E_1), 2397.50 ± 187.69 g (E_2). No significant differences in body mass between group C and the experimental groups were found. In the course of the experiment (0-8 weeks), rabbits in the control group (C) consumed a total of 7042.31 g per rabbit, or a daily average of 125.7 g per rabbit; rabbits in the test group E_1 consumed a total of 6673.24 g per rabbit, or a daily average of 119.16 g per rabbit; rabbits in the test group E_2 consumed 6699.72 g per rabbit, or a daily average of 119.63 g per rabbit. Food conversion (kg/kg) amounted to 4.13 in the controls, to 4.01 in group E_1, and to 3.92 in group E_2. Rabbits fed on 10% of olive cake, and particularly those consuming 20% of olive cake in their daily feed, manifested a better food conversion than rabbits in group C, whose fodder contained no olive cake.

Biochemical and haematological values of Hyla rabbits are shown in Table 4.

Table 4
Protein in rabbit serum at the end of the 56-day of experimental period

	Groups of animals		
n = 20	C (mean ± SD)	E_1 (mean ± SD)	E_2 (mean ± SD)
Total proteins g/l	66.09 ± 5.33	66.47 ± 8.40	65.84 ± 2.09
Albumin %	59.10 ± 3.04	58.34 ± 3.14	57.74 ± 3.39
Alpha$_1$ globulin %	2.33 ± 0.43	2.49 ± 0.61	2.41 ± 0.67
Alpha$_2$ globulin %	7.34 ± 0.74	7.43 ± 0.86	7.71 ± 1.00
Beta globulin %	14.54 ± 1.60	14.34 ± 1.51	14.87 ± 1.62
Gamma globulin %	16.63 ± 2.87	17.39 ± 2.71	17.44 ± 1.83

After 56 days of trial feed with different quantities of dried olive cake the concentration of total proteins in the serum of rabbits in all groups was found to be very similar, with no significant differences established between the groups.

The relative concentration of albumin in their serum, although the differences were not significant, were highest in group C, and lowest in group E_2.

Rabbits in both test groups had a relatively higher level of alpha$_1$ and alpha$_2$ globulin in their serum than did group C rabbits, although the established differences were not significant.

Rabbits in all 3 groups had a very similar relative proportion of beta globulin in the serum,

with the minimum and non-significant differences were established between the three groups.

Rabbits in groups E_1 and E_2 showed a higher relative participation of gamma-globulin in their serum that did those in group C, but again, the differences were not significant.

Table 5 shows concentrations of triacylglycerols, cholesterol, HDL and glucose in the serum of all groups.

Table 5
Concentration of triacylglycerols, total cholesterol, HDL and glucose in rabbit serum at the end of the 56-day experimental period

	Groups of animals		
n = 20	C (mean ± SD)	E_1 (mean ± SD)	E_2 (mean ± SD)
Triacylglycerols g/l	1.55 ± 0.47	1.57 ± 0.45	1.37 ± 0.51
Total cholesterol mmol/L	2.92 ± 1.09	**2.41 ± 0.60	**3.31 ± 0,97
HDL mmol/L	0.78 ± 0.20	**0.70 ± 0.18	**0.88 ± 0.23
Glucose mmol/L	7.94 ± 1.36	8.51 ± 2.35	8.61 ± 1.68

** = $P < 0.01$ (significant difference between experimental groups)

Rabbits in group E_2 showed a lower concentration of triacylglycerols in their serum than did those in group E_1 and group C with differences not significant.

After the 56-day period of trial feeding, significant differences were found in the concentration of total cholesterol (see Table 5) with group E_1 rabbits having the lowest concentration of total cholesterol lower ($P < 0.01$) than those of group E_2. At the same time, the ratio between concentrations of HDL was very similar to that of total cholesterol. Group E_1 rabbits had the lowest concentration of HDL in their serum lower ($P < 0.01$) than those in group E_2.

Rabbits in groups E_1 and E_2 tended to have higher, though not significantly, concentrations of glucose than those in group C.

Discussion

On the basis of achieved body weights it can be confirmed that the different proportions of olive cake added to feed had no adverse effect on the final body mass of fattened rabbits. Rabbits in group C consumed the largest quantities of feed, while the consumption of rabbits in both test groups (E_{1-2}) was similar and lower than that of group C. The ratio of food consumption is clearly visible from the average daily consumption in the course of the experiment. This data indicates that the quantities of dried olive cake (10% and 20%) used in a daily diet did not improve food consumption in fattened rabbits**.** Food conversion differed between the three groups. Rabbits in group C fed fodder containing no olive cake had the lowest level of food conversion. It was better in group E_1 (with 10% of olive cake), and best in group E_2 (with 20% of olive cake). It has to be pointed out that fodder for rabbits in group E_1 contained a slightly higher level of fat, whereas fodder for rabbits in group E_2 contained a considerably higher level of fat compared to that fed to rabbits in group C, which indicates that the fat content in the fodder, or rather its decrease or increase, reduced or improved food conversion, respectively, during the course of the fattening period. The energy value of all fodder mixtures used in the experiment was similar (see Table 2). At the end of the experiment, the serum of rabbits in all 3 groups showed very similar concentrations of total proteins, which points to the conclusion that the quantities (10 and

20 %) of dried olive cake used in the fattening process had no effect on the synthesis and transfer of serum proteins.

After 56 days of trial feeding, the highest relative proportion of albumin in serum was shown in group C, while in group E_1 a lower, and in group E_2 the lowest serum albumin proportion was found. Analysis of variance revealed no differences in the relative proportion of albumin among the three groups although there was a tendency to a decrease in proportion of serum albumin, especially in group E_2.

The relative proportion of $alpha_1$ and $alpha_2$ serum globulin was very similar among the groups, and the differences were non-significant. Similarly, no differences were detected among the 3 groups in the serum relative proportion of beta globulin. Rabbits in groups E_1 and E_2 had a slightly higher relative proportion of gamma globulin as compared to group C, although the differences were not significant. Addition of 10 and 20% olive cake thus did not affect the synthesis and the transfer of $alpha_1$, $alpha_2$, beta and gamma globulin in the serum of the animals.

Our values for total proteins in the serum of rabbits from all the groups were similar to the those found in healthy rabbits by Yu et al. (1979), Scheunert and Trautmann (1987), Bortolotti et al. (1989) and Lepitzki and Woolf (1991). Our relative values of the quantity of albumin in the serum of rabbits of all groups involved in the experiment were similar to the values quoted as physiological in rabbits by Irfan (1967), Yu et al. (1979) and Scheunert and Trautmann (1987), while they are higher than those given for healthy rabbits by Lepitzki and Woolf (1991).

The values of relative proportion of alpha globulin in the serum of rabbits of all groups in our experiment are lower than those found in rabbits by Irfan (1967), Scheunert and Trautmann (1987) and Bortolotti et al. (1989). Relative values of $alpha_2$ globulin are higher that those quoted by Scheunert and Trautmann (1987), and are the same as the relative values given by Irfan (1967) and Bortolotti et al. (1989).

The relative proportions of beta globulin in the serum of rabbits from all groups involved in our experiment were similar to the relative values of beta globulin found in rabbits by Irfan (1967) and Bortolotti et al. (1989), and slightly higher that the values given by Scheunert and Trautmann (1987).

The proportions of serum gamma globulin in rabbits of all groups in our experiment were similar to those described by Scheunert and Trautmann (1987), and are higher than the relative participation of gamma globulin given for rabbits by Irfan (1967) and Bortolotti et al. (1989).

To conclude, diets containing 10 and 20% of dried olive cake caused no disorders in the health state of rabbits with regard to total proteins and the proportions of individual serum proteins.

After 56 days of trial feed, rabbits from all groups in the experiment had approximately the same concentrations of triacylglycerols in their serum. Thus different quantities of dried olive cake, or rather, a different quantity and type of fats (olive oil - unsaturated fatty acids) in the feed had no influence on the content and transfer of triacylglycerols, i.e., on the lipogenesis of fattenned rabbits. Concentrations of triacylglycerols in the serum of rabbits in our experiment surpassed those of plasma triacylglycerols found by Yu et al. (1979), Leat (1983), Rupić et al. (1991) and Kortland et al. (1992).

The group E_1 rabbits (43.90 $g \cdot kg^{-1}$ of fats in the mixture), had a significantly lower serum total cholesterol concentration than those in group E_2, (52.95 $g \cdot kg^{-1}$ fats in the mixture). Thus the diet with 10% olive cake reduced the total serum cholesterol, whereas the diet with 20% increased significantly this parameter in group E_2.

A higher fat amount in the feed (by 10.45 $g \cdot kg^{-1}$) resulted in an elevated total cholesterol concentration in the serum of rabbits in group E_2 caused, no doubt, by the type of fat - olive oil from the dried olive cake. Values of the serum total cholesterol found in our rabbits are

higher than those found in rabbits by Yu et al. (1979), Caisey and King (1980), Lupa and Calb (1988), Lepitzki and Woolf (1991) and Kortland et al. (1992). They are within the limits of the physiological values given for rabbits by Scheunert and Trautmann (1987), but are considerably lower than the values found in healthy rabbits by Morrisett et al. (1991).

The ratio of serum concentrations of HDL between all groups of rabbits were very similar to that of total cholesterol concentration. Group E_1 rabbits showed the lowest concentration of HDL, and significantly lower than the rabbits in group E_2. Cholesterol from HDL (alpha-lipoprotein) is not harmful; it has a protective effect and prevents the onset of coronary diseases. HDL assists in removing surplus peripheral cholesterol by binding it, directing it to the liver where it is catabolized, and excreted with bile (bile salts). Our data show that with an increased fat content in feed (52.95 $g \cdot kg^{-1}$ at 20% dried olive cake), the quantity of HDL was higher than with 10% olive cake diet, or 43.90 $g \cdot kg^{-1}$. It is concluded that dietary lipids, in particular those from olive cake (olive oil), influenced the quantity of HDL in the serum of our experimental rabbits. The increased concentration of HDL indicates a more rapid elimination of harmful cholesterol from the body, which prevents the onset of pathological processes in blood vessels, particularly in the heart. In relation to total cholesterol in group C rabbits, (2.92 mmol/L), HDL accounts for 26.71%, in rabbits from group E_1 (2.41 mmol/L) for 29.05%, while for rabbits in group E_2 (3.31mmol/L) the figure is 26.59%. In the research carried out by Vrecko et al. (1988) HDL accounted for 62.1 - 74.65%, while Bortolotti et al. (1989) found that HDL accounts for 52 ± 6% of total cholesterol in the serum of healthy rabbits. Values for HDL in the rabbits of all groups involved in the experiment are considerably lower in relation to those found by the quoted authors. To conclude the discussion on the overall cholesterol in the serum we wish to point out that we obtained the results we expected with regard to the concentration of total cholesterol in the serum, since rabbits in group E_2 consumed 25.5% more fat than those in group C. However, we did not anticipate such a low concentration of HDL in rabbits in group E_2. If one bears in mind that rabbits in group E_2 consumed food which contained considerably higher quantities of olive oil, or rather, of unsaturated fatty acids (linolic, linolenic), which bear a significant influence on the quantity of HDL in the serum, we would have expected the concentration of HDL to be much higher in the serum of rabbits in group E_2 in comparison with those in group C, whose feed contained no unsaturated fatty acids.

Rabbits in groups E_1 and E_2 had a similar, although slightly higher concentration of glucose in their serum than those in the control group. The results obtained show that after 56 days of trial feed, different quantities (10 and 20%) and chemical compositions of dried olive cake did not influence the concentration of glucose in rabbit serum. Values of glucose concentration in all groups of rabbits are higher than those found in rabbit serum (plasma) by Yu et al. (1979), and as mentioned by Scheunert and Trautmann (1987) and Hewitt et al. (1989). They are, however, very similar to the values found in rabbits by Fox and Laird (1970), Nordestgaard and Zilversmit (1988) and Lepitzki and Woolf (1991).

Our experiment demonstrated that the concentrations of proteins, their fractions, triacylglycerols, total cholesterol, HDL and glucose in the serum of rabbits were not adversely affected by addition of 10% and 20% dried olive cake. The synthesis and transfer of proteins and fats in the body was not impaired. In conclusion, dried olive cabek may be included in diets for rabbits.

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