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REGULAR ARTICLE

EFFECT OF USING SATURATED AND UNSATURATED FAT WITH MIXING THEM IN BROILER DIET ON BLOOD PARAMETER

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ABSTRACT

In this work we investigated the effect of three types of fat substances based on the use of 5% packed fat (PF) as saturated fat (SF) for control group and 2.5%PF+2.5% rapeseed for treatment1. 2% PF+2.5% sunflower oil as unsaturated fat (USF) and also mixing between them with different level proportion in the diet of broiler (*Ross-308*) chickens. Lower cholesterol levels in the blood were observed in groups T1 for male and female (3.83, 3.37 Mmol.l^{-1}) respectively compared to other groups. The results show that lowest group for triglyceride for female was in group T2 (0.33 Mmol.l^{-1}) and for male was in Cgroup (0.34Mmol.l^{-1}). High level of total protein in blood for female was in group T3 (37.04g.l^{-1}) and for male was in group T2 (48.35g.l^{-1}). Differences were significant (P≤0.01).

Keywords: broiler (Ross-308), blood cholesterol, triglyceride, total protein

INTRODUCTION

There were many studies of effect of fat in diet on blood cholesterol and other parameters of serum blood. **Aggoor et al. (2000)** evaluated three fat sources (SO, PO or ultra cal composed of a mixture of soybean oil, corn oil, rapeseed oil and high oleic palm oil) at levels of 2.5% or 5.0% in broiler diets. They found, that the three concentrations of plasma

total lipids, triglycerides and cholesterol were increased concurrently with increasing the dietary fat levels, while they were not affected by the studied sources. **Crespo and Esteve Garcia (2003)** showed that addition of PUFA with rich fats to diets reduced serum cholesterol of broiler chicks.Several dietary components have been shown to affect serum cholesterol concentrations in a wide variety of species. Conclusions have varied regarding to the effect of dietary fat of animal origin on one hand and of a plant origin on the other.

Badway (1997)recognized that the supplementation of the rations with 5% sunflower oil resulted in a reduction of triglyceride concentrations on blood with about 40% compared to the control. She added that increasing the supplementation levels of sunflower oil to 10% resulted in a significant increase in the triglyceride levels. A 23.3% proportion tototal lipids is blood. **Abdul Raufe (2007)** found, that the addition of corn oil to diet rations decreased blood total lipids during the growing and laying period compared with animal fat rations. The decrease was more pronounced by increasing corn oil levels from 3% to 6 %. Interest in dietary fat andCoronary heart disease(CHD) was centered primarily on saturated and polyunsaturated fatty acids until 1985, when **Grundy (1986)** reported, that monounsaturated fatty acids, namely oleic acid, were aseffective as PUFA in reducing plasma total and LDL cholesterol levels. These observations coincided with the relatively low incidence of CHD observed among populations consuming the so-called “Mediterranean diet,” which is characterized by a high intake of fat but primarily from olive oil. The prevailing theory at the time argued, that saturated fatty acids raised blood cholesterol, PUFA lowered blood cholesterol and MUFAs were neutral, they neither raised nor lowered blood cholesterol **(Abdel-Hakim et al., 1982).** The demonstration showed that vegetable oils with high concentration in oleic acid were effective in reducing blood cholesterol **(Vilchez et al., 1991).**Transfatty acids are produced, when fats and oils are hydrogenated (hardened) for use in the manufacture of margarines and derivative. The report by **Kinsella et al. (1990)** that high intakes of transfatty acids not only increased plasma LDL cholesterol levels, but lowered plasma highdensity lipoprotein (HDL) cholesterol levels, triggered an intense debate on the physiological effects of hydrogenated fats, particularly in relation to CHD.

The objective of our study was to find effect of each type of fat SF and USF with mixing them on blood parameters which reflect on muscles properties.

MATERIAL AND METHODS

The experiment was realized at the test station poultry of Slovak Agricultural University in Vígľaš; research farmon feeding of Ross 308 chicken hybrid combination. The experimentenrolled 800 pieces of one day chickens hybrid combination and were created 4 groups ofanimals: control (C) and three experimental (I, II and III) of 50 pcs of chickens. Custom feedinginsisted 42 days. Chickens are housed in the experimental procedure under the same technological conditions. Viewed climatic variables must meet the criteria for the type and category of animals. Other technology systems (ventilation, lighting intensity, length of day light)implemented as recommended by the fattening technology applicable to a particular hybrid chicken included in the experiment.

The feed formulation and feeding periods

Isocaloric and isonitrogenous diets formulated by the use of the program (G7 2000) are based on least cost design.

Feeding by compound feed

Feed mixture will be loose fed, without other feed ad libitum. Fresh, hygienic drinking water will still be available from automatic drinker. Production, sampling and analytical were assessment the feed mixtures. Feed mixture will be produced under the supervision of staff research team at the BTS Vígľaš; the production will be equalized in bags.Compound feed production will be sampled after taking subsamples for bagging. Thesub-sampleswill be prepared bulk sample, which will be adjusted to an appropriate reduction in the size of laboratory samples. Feed should be sampled official sampling procedure under Regulation (ES). 152/2009 laying down methods of sampling and analysis for official controls on feed.Analytical evaluation of the feed will be carried out in laboratories of the Central Control and Testing Institute in Agriculture (ÚKSÚP) Zvolen, in scope as stated in the "Viewed indicators.Recipes complete feed mixtures were designed by ÚKSÚPu, sponsor experiment suggested dosage product verification. To calculate the nutritional value of compound feed materials were used with the following content of crude protein: 70.0 g maize / kg, 107.1 g wheat / kg, soybean meal 460.4 g / kg and 619.5 g fishmeal / kg. Feed formula shall be adjusted for the analytical determination of individual nutrients in feed materials, if necessary premix additives. In developing the final formula will be followed so that the compoundmeets therequirements of type- Slovakia government under Regulation

number. 440/2006 on feed mixtures in compliance with the experimental intervention consisting of doses of fat and vegetable oilswith incorporating Packed Fat, Sunflower oil and Rapeseed oil into broiler starter and diet. The feeding duration is 7 days for prestarter, 9 days for starter, 17 days for grower, and 5 days for finisher. Diets were provided ad libitum. The feed mixtures formulated for each period of feeding and chemical analysis of the feed mixtures presented in tables 1, 2, 3, and 4.

Table 1 Pre-starter feed mixtures formula and chemical analysis

Components		Groups			
		%			
		C	T1	T2	T3
Maize	%	44.20	44.20	44.20	44.20
Soybean meal		32.00	32.00	32.00	32.00
Wheat		10.00	10.00	10.00	10.00
Fishmeal		5.00	5.00	5.00	5.00
Limeston($Ca\ Co_3$)		1.35	1.35	1.35	1.35
monocalcum phosphate		1.00	1.00	1.00	1.00
*PX BR Unit		1.00	1.00	1.00	1.00
Methionen 99%		0.12	0.12	0.12	0.12
Total salt		0.20	0.20	0.20	0.20
Therionine 99 %		0.13	0.13	0.13	0.13
Backed fat		5.00	2.50	2.50	2.50
Sunflower oil		-	2.50	-	1.25
Rapeseed oil		-	-	2.50	1.25
TOTAL		100.00	100.00	100.00	100.00
Chemical composition					
Crude protein	%	23.69	23.17	23.36	23.48
Crud fat		7.57	8.03	8.09	8.23
Crude fiber		2.8	2.6	2.9	2.9
Ash		6.31	6.43	6.45	6.33
Calcium		11.023	11.920	11.582	11.107
Total phosphor	$mg.kg^{-1}$	7.417	7.500	7.417	7.500
Sodium		18.1	18.2	19.0	18.5
Magnesium		2.202	2.326	2.268	2.238
ME_N	$MJ.Kg^{-1}$	12.702	12.946	12.811	13.140

Legend : *vit. A=4,500,000 IU, vit. D=1,660,000 IU, vit. E=20,000 $mg.kg^{-1}$, vit. K3=1, $mg.kg^{-1}$, vit. B1=1,800 $mg.kg^{-1}$, vit. B2=2,500 $mg.kg^{-1}$, vit. B6=1,600 $mg.kg^{-1}$, vit. B12=8.75 $mg.kg^{-1}$, folic acid=600 $mg.kg^{-1}$, calcium pentonite=5,500 $mg.kg^{-1}$, niacinamid=18,000 $mg.kg^{-1}$, biotin=60 $mg.kg^{-1}$, cholin clorid=30,000 $mg.kg^{-1}$, betain=65,000 $mg.kg^{-1}$, cobalt=150 $mg.kg^{-1}$, Iodine=380 $mg.kg^{-1}$, Mn=45,800 $mg.kg^{-1}$, cupper=6,500 $mg.kg^{-1}$, Si=110 $mg.kg^{-1}$, Zn=28,300 $mg.kg^{-1}$,Fe=27,200$mg.kg^{-1}$, Mo=350 $mg.kg^{-1}$.

Table 2 Starter feed mixtures formula and chemical analysis

Components		Groups			
		%			
		C	T1	T2	T3
Maize	%	48.50	48.50	48.50	48.50
Soybean meal		29.00	29.00	29.00	29.00
Wheat		10.00	10.00	10.00	10.00
Fishmeal		4.00	4.00	4.00	4.00
Limeston($Ca\ Co_3$)		1.30	1.30	1.30	1.30
monocalcum phosphate		0.85	0.85	0.85	0.85
PX BR Unit		1.00	1.00	1.00	1.00
Methionen 99%		0.05	0.05	0.05	0.05
Total salt		0.22	0.22	0.22	0.22
lysine		0.03	0.03	0.03	0.03
Therionine 99 %		0.05	0.05	0.05	0.05
Backed fat		5.00	2.50	2.50	2.50
Sunflower oil		-	2.50	-	1.25
Rapeseed oil		-	-	2.50	1.25
TOTAL		100.00	100.00	100.00	100.00
Chemical composition					
Crude protein	%				
Crud fat		22.01	21.46	21.76	22.06
Crude fiber		7.63	8.91	7.88	8.00
Ash		2.3	3.0	2.4	2.2
Calcium		5.94	6.93	6.01	5.98
Total phosphor	$mg.kg^{-1}$	10.943	13.772	10.785	10.665
Sodium		7.500	7.417	6.917	7.084
Magnesium		18.10	18.20	19.00	18.50
ME_N	$MJ.Kg^{-1}$	2.236	2.499	2.143	2.227
		12.950	13.057	13.052	13.208

Table 3 Grower feed mixtures formula and chemical analysis

Components		Groups %			
		C	T1	T2	T3
Maize	%	42.40	42.40	42.40	42.40
Soybean meal		29.00	29.00	29.00	29.00
Wheat		20.00	20.00	20.00	20.00
Limeston(Ca Co_3)		1.35	1.35	1.35	1.35
monocalcum phosphate		0.80	0.80	0.80	0.80
*PX BR Unit		1.00	1.00	1.00	1.00
Methionen 99%		0.05	0.05	0.05	0.05
Total salt		0.33	0.33	0.33	0.33
lysine		0.02	0.02	0.02	0.02
Therionine 99 %		0.05	0.05	0.05	0.05
Backed fat		5.00	2.50	2.50	2.50
Sunflower oil		-	2.50	-	1.25
Rapeseed oil		-	-	2.50	1.25
TOTAL		100.00	100.00	100.00	100.00
Chemical composition					
Crude protein	%	19.28	19.89	19.20	19.35
Crud fat		7.36	7.54	7.41	7.64
Crude fiber		2.8	3.0	3.0	2.5
Ash		5.76	5.63	5.63	5.67
Calcium		10.943	13.772	10.785	10.665
Total phosphor	$mg.kg^{-1}$	7.500	7.417	6,917	7.084
Sodium		16.00	15.50	16.70	16.30
Magnesium		2.236	2.499	2,143	2.227
ME_N	$MJ.Kg^{-1}$	1.3	1.3	1.3	1.3

Table 4 Finisher feed mixtures formula and chemical analysis

Components		Groups %			
		C	T1	T2	T3
Maize	%	40.50	40.50	40.50	40.50
Soybean meal		22.60	22.60	22.60	22.60
Wheat		28.00	28.00	28.00	28.00
Limeston($Ca\ Co_3$)		1.35	1.35	1.35	1.35
monocalcum phosphate		0.80	0.80	0.80	0.80
*PX BR Unit		1.00	1.00	1.00	1.00
Methionen 99%		0.20	0.20	0.20	0.20
Total salt		0.30	0.30	0.30	0.30
lysine		0.15	0.15	0.15	0.15
Therionine 99 %		0.10	0.10	0.10	0.10
Backed fat		5.00	2.50	2.50	2.50
Sunflower oil		-	2.50	-	1.25
Rapeseed oil		-	-	2.50	1.25
TOTAL		100.00	100.00	100.00	100.00
Chemical composition					
Crude protein	%	17.73	17.86	17.61	17.76
Crud fat		7.30	7.26	7.51	7.37
Crude fiber		2.5	2.4	2.8	2.4
Ash		5.39	5.39	5.44	5.45
Calcium		10.366	10.739	10.467	10.273
Total phosphor	$mg.kg^{-1}$	6.367	6.100	5.834	6.00
Sodium		14.4	14.2	13.5	15.8
Magnesium		2.293	2.296	2.285	2.236
ME_N	$MJ.Kg^{-1}$	1.3	1.3	1.3	1.3

Trial management

Broiler chickens were kept under the Ross recommended procedure. Water and rations distributed *ad libitum* and uniform light provide 24 hours daily. The temperatures ofthe house and vaccination programme applying are basing on broilerlive breeding period raisers' recommendations. Chickens in the course of the trial were housed on the deep litter in the same technological conditions. Microclimate indicators in the range of temperature and humidity were measured and recorded three times a day, at 7.00 am, 12.00 and 17.00 pm. Measurement indicated in the zone of animals, in the height from the floor, where the largest part of the body of animals.

Biochemical parameters for blood

The blood serum was used for evaluation of selected parameters of fat metabolism (cholesterol, triglycerides) and mineral profile (calcium, inorganic phosphorus). In this study the blood serum of broilers was analyzed in order to find the effect of natural additives when given in the diet. At 32 days old of the chickens, four blood serum samples from each replicate of treatment were collected. Blood samples were taken after the slaughtering into disposable heparinised test tubes. Prior to freezing at -18°C, these blood samples centrifuged at 3000 rpm for 10 minutes, and a commercial instrument (Labofuge 300 – Heraeus) was used to collect the blood serum. Mineral profile (calcium, phosphorus, magnesium, potassium and sodium) and energy with enzymatic profile. Total cholesterol, Triglycerides and total protein were analytic.

Statistical analysis

For the statistical design and data analyses, complete random design an experiment with 4 treatments were determined. Data in all experiments were subjected to ANOVA procedures appropriate for a completely randomized design and the significance of differences between the means estimated using Duncan test (Duncan's new multiple range test). Probability level of was Significance in all comparisons with chemical parameters which $P<0.01$ was considered. Values in percentage were subjected to transformation of Arc sin v100. All statistical analyses were performed using the software SPSS 17.5 for Windows® (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The determination of blood component values using laboratory exams is an important procedure to give inputs for research studies on nutrition, physiology, and pathology **(Bounous et al., 2000).** The interaction of minerals is also an important factor. Well known is the close interaction of P and Mg in broilers **(Gaal et al., 2004).** The concentration of avian blood lipids is influenced by the physical and nutritional status of the bird **(Abdel-Fattah et al., 2008).**

Serum biochemical data of broiler chickens are showed in table 5. The treatments had little influence on biochemical parameters.

The serum total cholesterol and triglyceride level did not differ significantly among treatment groups excepted T2 group for a triglyceride male. **Anitha et al. (2007)** revealed that insignificant differences of total cholesterol and triglyceride were found between groups when broilers fed diets contain different levels of rise brain oil. Our findings on blood cholesterol are in agreement with reports of **Lee et al. (2003)** and **Abdel-Fattah et al. (2008).** Cholesterol was increased in the group receiving mixing in diet with equal percentage of sunflower oil and packed fat when compared to the C group. Lower levels for both sexes were found in T1 followed by C group compared to T2 and T3. This is may be due to the organic acids contained in the diet of those groups and MUFA enrichment, compared to control. **Abdo (2004)** found that blood cholesterol decreased significantly by dietary acidifiers. There is increasing evidence that dietary monounsaturated fatty acid enrichment decreasing low-density lipoprotein cholesterol but not high-density lipoprotein cholesterol in blood plasma, and decreasing the susceptibility of low-density lipoprotein to oxidation **(Roche, 2001).**

Triglyceride levels were insignificant differences ($P>0.01$) for female and the lower in groups T2 compared to other groups. These results suggesting high activity of lipid metabolism in the body for the groups received mixing sunflower oil with packed fat which was parallel with the low fat deposition in the abdomen in latest groups, in the other hand, high PUFA intake of those groups leading to greater substrate availability and thus faster b-oxidation of polyunsaturated fatty acids **(Leyton et al., 1987)** thus, reduced triglyceride.

Also, may be the lower feed consumption, during the early period of breeding (pre-starter), and consequently lower fat intake that resulted in fat depletion may also contribute in reducing blood lipid content. **Pinchasov and Jensen (1989)** and **Zhang et al. (2005)** observed an inhibition of feed intake in chicken due to chemical feed restriction induced by supplemental organic acids. Moreover, the observed hyperthyroidism associated with dietary organic acidification could also explain the observed reduction in serum lipid profile. In addition, the beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions **(Young and Foegeding, 1993).**

Table 5 observed there were significant differences ($P<0.01$) for triglyceride in male chicks and high value was in T2 (0.78 $Mol.l^{-1}$) followed by T3, T1 and C groups respectively. On the other hand for female estimation of triglyceride, there were insignificant differences ($P>0.01$), but there were arithmetic differences and high value was equal in C and T3 groups

while low value was in T2. This can be attribute by affect of sex by androgen hormone lead to accumulated of triglyceride in blood.

Total protein was significant differences ($P<0.01$) for male and high value was T2 (48.35 $g.l^{-1}$)followed by T3, C and T1 groups respectively. On other handtable 5 observed there were insignificant differences ($P>0.01$) for female and the low value estimated in C group (34.4$g.l^{-1}$). This can explain by there is opposite relation between type of fat and proportion of total protein and moisture in tissues **(Mohammed et al., 2005).**

Table 5 Mean ±Sd of biochemical data of serum samples at 42 days of age from broiler chickens submitted to different treatments containing differs type of fat in different levels

Attributes	Units	Groups							
		C		T1		T2		T3	
		Female	Male	Female	Male	Female	Male	Female	Male
Cholesterol	$Mmol.l^{-1}$	3.54±0.22	3.91±0.63	3.37±0.13	3.83±0.31	3.67±0.45	4.33±0.24	3.60±0.23	4.10±0.99
Triglyceride	$Mmol.l^{-1}$	0.46±0.18	0.34±0.04 [a]	0.39±0.08	0.35±0.08 [a]	0.33±0.04	0.78±0.18 [b]	0.46±0.10	0.37±0.10 [a]
Total protein	$g.l^{-1}$	34.4±1.31	33.83±3.2[a]	35.10±3.1	32.93±3.6[a]	36.53±3.34	48.35±5.1[b]	36.55±4.42	37.04±5.64 [a]

CONCLUSIONS

From present results we conclude:

1. The blood parameters influenced negatively but without any negative effects on overall performance.
2. Group which supplied by packed fat SF mixed with sunflower oil USF lead to increase levels of cholesterol in blood.
3. Group which supplied just SF high light level of triglyceride. This will reflect on metabolic of muscle meat which have role for human health.
4. There were opposite relationship between levels of SF and proportion of protein in chicks' blood.

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