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RESEARCH ARTICLE

Effects of Various Fat Sources Added into the Diets of Laying Hens on Calcium and Phosphorus Metabolism

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ABSTRACT

This research was conducted to determine the effects of different vegetable oils and animal fat added into laying hens' rations at different levels during the late laying period on serum calcium and phosphorus levels. A total of 54 sixty-seven weeks old Isa Brown hens were weighed and randomly distributed into nine dietary treatment groups as C (control fed with only basal diet), T2 (basal diet + 2% tallow), M2 [basal diet + 2% tallow and linseed oil mixture (50/50)], S2 (basal diet + 2% sunflower oil), L2 (basal diet + 2% linseed oil), T4 (basal diet + 4% tallow), M4 [basal diet + 4% tallow and linseed oil mixture (50/50)], S4 (basal diet + 4% sunflower oil) and L4 (basal diet + 4% linseed oil), respectively. Each treatment group consisted of 6 subgroups, comprising of 1 bird each. At the end of the study, serum calcium and phosphorus levels (mg/dl) of C T2, M2, S2, L2, T4, M4, S4, L4 groups were 18.63 and 5.85, 18.03 and 4.52, 18.60 and 5.00, 16.62 and 4.42, 17.55 and 4.33, 16.25 and 5.63, 17.48 and 3.95, 17.47 and 3.28, and 21.43 and 5.12, respectively. The highest calcium level was observed in L4 group (21.43 mg/dl), while the highest phosphorus level (5.85 mg/dl) was detected in the control (C) group. As a result of the research, no significant effect ($p>0.05$) was found in terms of different fat sources and levels among the groups during the late laying period on the serum calcium and phosphorus levels of laying hens.

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1. Introduction

The poultry sector is an important branch of animal husbandry in Türkiye. According to the latest data of TÜİK (2021), 35% of poultry poulation consists of laying hens and approximately 20 billion (1.2 million tons) eggs are produced annually. However, the inadequacy of animal feed resources in terms of both quantity and quality is often the biggest obstacle to the development of this sector (Ergün & Bayram, 2021; Kaya et al., 2019). To maintain their productivity healthly and to produce quality products without loss, the nutrients needed by laying hens must be provided in sufficient quantities and in usable form with diets.

Ca and P are the main structural elements of the skeletal system in animals and are indispensable for the normal course of vital functions. Due to the relationship between them, Ca and P are handled together in animal nutrition and the ratio is desired to be 2:1 or 3:1 for optimum utilization. In addition to being structural elements, Ca and P, which exist as ions and other compounds, play a role in many physiological processes (Matuszewski et al., 2020). Ca, the main component of bone tissue and egg shell production, is essential for poultry due to

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its role in acid-base balance and enzymatic system. It affects eggshell quality, bone mineralization and growth (Li et al., 2020). Many researches have shown that the shell of each egg contains 2.2 g of calcium. Deficiency in Ca causes deformation in the skeletal system, tibial dyschondriplasia, rickets, nerve damage and a decrease in egg production and its shell quality (Xing et al., 2020). Also, P is an important element in living organisms beceouse of metabolic functions. It plays a big role in phosphorylation reactions in biological systems such as synthesis of some energetic compounds like ATP, ADP, phosphocreatine and similar metabolites in addition to formation of bone and egg shell, acid-base balance, carbohydrate, lipid and protein metabolism (Çelebi et al., 2005).

Ca and P concentrations in the body and their status in stores have a dynamic structure and are subject to regulation. Due to this regulation through hormonal control, bone mineral content constantly changes. This Ca-P exchange between tissues and stores is particularly important in high-producing animals such as dairy cows and laying hens, where the need for these minerals is several times higher than the other animals (Matuszewski et al., 2020).

Egg shell quality problems in laying hens are important and increase with age. In many feeding studies conducted to improve shell quality, it has been reported that the factors affecting bioavailability are as important as the amount and sources of calcium and phosphorus (Bintaş & Özdoğan, 2017).

Oils are routinely added to mixed feeds in today's feed industry because they are a cheap energy source, prevent dust formation, reduce heat stress and have many other important benefits (Çelebi & Macit, 2008; Kurt & Küçük, 2010).

Fats used to increase efficiency in poultry rations provide a significant portion of dietary energy, as well as being a source of other nutrients such as vitamins. However, there are many research evidences that fats or oils have positive or negative effects on the digestion and absorption of inorganic substances in animal nutrition (Kurt & Küçük, 2010). Studies have shown that oils rich in unsaturated fatty acids are better digested and absorbed by poultry than saturated fats (Atteh & Leeson, 1984; Leeson & Atteh, 1995). Since oils are water-insoluble compounds, an emulsion step is necessary for their absorption. Because of phospholipid group of oils has surface-active properties, they are important in the emulsification of lipids and can affect the absorption of nutrients in the small intestine (Huang et al., 2007). Indeed, previous studies on poultry (Atteh & Leeson, 1984) have shown that soap formation occurs from dietary fatty acids with minerals during the digestion process, and that the level of digestion and absorption of soap significantly affects the utilization of the relevant fatty acids. Atteh and Leeson (1985) also observed that increasing the calcium content of the diet aggravated the soap formation problem.

In the presence of fats, the absorption of calcium from the digestive system is partially inhibited. Because Ca^{++} ions and fatty acids form insoluble soaps, which negatively affects the utilization of both calcium and fats. If fat is added to poultry rations, it is necessary to increase dietary calcium to improve feed conversion rate (Tabeidian et al., 2010; Utlu & Çelebi, 2004).

Another important factor affecting digestion is whether fats are saturated or unsaturated. There are studies showing that unsaturated fats are better used than saturated fats in animal metabolism and have higher ME (Çelebi & Macit, 2008).

The main factors affecting the digestion and absorption of fats in chickens are the fatty acid composition of the dietary fat, the mineral content of the diet, especially calcium, and the type, breed and age of the poultry (Atteh & Leeson, 1985; Baucells et al., 2000). Examining the effects of age on fat utilization in poultry, Duckworth et al. (1950) found that the ability of chicks to digest tallow increased with age up to 4 weeks. In another study, Renner and Hill (1960) observed that tallow could be used in 8-week-old chickens as much as in adult chickens. Similarly, Fedde et al. (1960) determined that 1-week-old chicks could digest beef tallow at a rate of 53%, and this rate increased to 80% when they were 12 weeks old. On the other hand, Whitehead and Fisher (1975), who investigated the effect of fat type and animal age on the evaluation of fats, observed that the digestibility of tallow was 57% in 2-weeks-old chicks and 74% in 8-weeks-old chickens, while they reported that there was no difference in the use of corn oil and lard in old hens.

Additionally, apart from the studies conducted with vegetable oils and animal fats, many recent studies showed that the addition of essential oils to poultry diets in even very small amounts increases serum Ca and P levels by reducing the pH of the digestive system increasing the intestinal surface area and the amount of endogenous enzymes (Sevim et al., 2020).

In present study, it was aimed to determine whether the inclusion of various vegetable and animal fat sources with different degrees of saturation in the diet at different levels affects serum calcium and phosphorus levels, in order to provide a solution to the egg shell problems that increase with age in laying hens.

2. Materials and Methods

The data of this study was obtained from the laying hens reared at the Research and Application Farm of the Agricultural Faculty, Atatürk University. Isa Brown layers (n=54, 67 weeks of age) were weighed and randomly assigned to nine dietary treatment groups and reared in individually cages (50 cm × 46 cm × 46 cm; length × width × height). Each treatment group was replicated six times as subgroups, comprising of 1 bird each. The layers at the late laying period were fed once daily with an isonitrogenous but not isocaloric commercial layer feed for 8 weeks. As shown in Table 1, control group was fed with basal diet (C) containing about 16 % CP, 2650 kcal ME/kg and 3.77% Ca and 0.29% available P. Feeds and water were offered *ad-libitum* and all hens were exposed to 16 h of light day^{-1}.

The experimental diets were made by adding the sunflower, linseed and tallow fats and their mixture (w/w) to the basal diet at 2% and 4% levels. Control group was fed with only basal diet, the experimental groups were fed with basal diet + 2% tallow (T2), basal diet + 2% mixture of tallow and linseed oil (w/w) (M2); basal diet + 2% sunflower oil (S2), basal diet + 2% linseed oil (L2), basal diet + 4% tallow (T4), basal diet + 4% mixture of tallow and linseed oil (w/w) (M4), basal diet + 4% sunflower oil (S4), and, basal diet + 4% linseed oil (L4). Table 1 shows the ingredients and chemical composition of experimental diets. The basal diet was formulated according to the recommendations of the National Research Council (NRC, 1994; Table 1). At the end of the eight weeks feeding period, blood samples for determination of plasma Ca and P values were obtained from the wing veins of six hens per treatment, the plasma was separated by centrifugation blood for 10 min. at 2000 x g and stored at -20 °C for further measurements. The Ca and P were analyzed on auto analyzer by using commercial kits.

Table 1. Ingredients and chemical composition of basal and experimental diets fed to laying hens.

	Experimental groups[1]								
Ingredients (%)	**C**	**T2**	**M2**	**S2**	**L2**	**T4**	**M4**	**S4**	**L4**
Corn	38.30	37.30	37.30	37.30	37.30	36.30	36.30	36.30	36.30
Wheat	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Barley	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean meal	12.00	13.00	13.00	13.00	13.00	14.00	14.00	14.00	14.00
Full Fat soybean	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Meat-bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	-	2.0	-	-	-	4.0	-	-	-
Tallow and linseed oil (w/w)	-	-	2.0	-	-	-	4.0	-	-
Sunflower oil	-	-	-	2.0	-	-	-	4.0	-
Linseed oil	-	-	-	-	2.0	-	-	-	4.0
Marble meal	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine-HCl	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Chemical Analysis									
ME (kcal kg^{-1})[3]	2650	2800	2800	2800	2800	2950	2950	2950	2950
Crude protein (%)	16.0	16.07	16.07	16.07	16.07	16.10	16.10	16.10	16.10
Crude fiber (%)	4.63	4.68	4.68	4.68	4.68	4.70	4.70	4.70	4.70
Crude ash (%)	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40
Ether extract (%)	2.70	4.65	4.65	4.65	4.65	6.60	6.60	6.60	6.60
Ca (%)	3.77	3.77	3.77	3.77	3.77	3.77	3.77	3.77	3.77
P (%)	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29

[1]C: Control, basal diet; T2: basal diet + 2% tallow; M2: basal diet + 2% mixture of tallow and linseed oil (w/w); S2: basal diet + 2% sunflower oil; L2, basal diet + 2% linseed oil, T4: basal diet + 4% tallow; M4: basal diet + 4% mixture of tallow and linseed oil (w/w); S4: basal diet + 4% sunflower oil; L4, basal diet + 4% linseed oil.
[2]Each 2.5 kg vitamin premix contains: Vitamin A, 8.000.000 IU; vitamin D3, 2.000.000 IU; vitamin E, 20 mg; K3, 3000 mg; B1, 1500 mg; B2, 4000 mg; B12, 500 mg; nicotinamide, 6000 mg; Ca D-pantothenate, 6000 mg; B6, 2500 mg; choline-Cl, 200 000 mg; biotin, 1000 mg.
[3]Calculated values.

2.1. Statistical Analysis

All data were analyzed with SPSS (1999) statistical software package for Windows 10.0 version by using one-way analysis of variance (ANOVA).

3. Results and Discussion

The results related to the effects of the inclusion of different fat sources at different levels to diets of laying hens on serum Ca and P levels are presented in Table 2. All literature reports regarding serum Ca and P levels presented here are expressed as mg/dl.

Table 2. The effect of different fat sources in diets on serum parameters of laying hens.

Treatment Groups	Parameters	n	Serum (mg/dl) Ca $\bar{X} \pm S\bar{x}$	P $\bar{X} \pm S\bar{x}$
C	Control (only basal diet)	6	18.63 ± 025	5.85 ± 0.54
T2	BD + 2% tallow	6	18.03 ± 0.46	4.52 ± 0.54
M2	BD + 2% mixture (1% T + 1% L)	6	18.60 ± 0.42	5.00 ± 0.77
S2	BD + 2% sunflower	6	16.62 ± 0.89	4.42 ± 0.57
L2	BD + 2% linseed	6	17.55 ± 0.33	4.33 ± 0.38
T4	BD + 4% tallow	6	16.25 ± 1.11	5.63 ± 1.10
M4	BD + 4% mixture (2% T + 2% L)	6	17.48 ± 0.91	3.95 ± 0.79
S4	BD + 4% sunflower	6	17.47 ± 1.50	3.28 ± 0.62
L4	BD + 4% linseed	6	21.43 ± 2.35	5.12 ± 0.73
Significance			*ns*	*ns*

C: Control, fed with only basal diet, T2: Basal diet + 2% tallow, M2: Basal diet + 2% tallow and linseed oil mixture (50/50), S2: Basal diet + 2% sunflower oil, L2: Basal diet + 2% linseed oil, T4: Basal diet + 4% tallow, M4: Basal diet + 4% tallow and linseed oil mixture (50/50), S4: Basal diet + 4% sunflower oil, and L4: Basal diet + 4% linseed oil, BD: Basal diet, ns: non significant, ($p>0.05$).

The inclusion of various vegetable oil and animal fat sources, having different degrees of saturation or unsaturation, to the diets of aged hens during the late laying period did not affect investigated serum parameters. There were no significant ($p>0.05$) differences among the groups in terms of serum Ca and P levels.

In contrast to the current study, Bölükbaşı et al. (2005) investigated the effects of dietary Ca and vitamin D_3 supplementation on plasma Ca and P levels of late-laying hens and determined that plasma Ca (9.7-17.07) and P values (3.35-4.95) were affected ($p<0.01$) by dietary treatments.

Smilarly, Utlu and Çelebi (2004) stated that adding sunflower oil at different levels (0, 2, 4 and 6%) to the young goose (goslings) diets did not have significant effect on serum Ca (10.5-11.7) and P (8.9-10.5) levels. The findings of the research were compatible with the results of present study.

As shown in Table 2, the group fed with diet including 4% linseed oil showed the highest serum Ca level (21.43). On the other hand, the highest serum P level (5.85) was found in the control group. It was observed that by increasing the amount of saturated fat in the diet, serum Ca levels decreased (T2: 18.02 vs. T4: 16.25), and on the contrary, with the addition of highly unsaturated vegetable oils to diet serum Ca levels (S4: 17.47 vs S2: 16.62 and L4: 21.43 vs L2: 17.55) increased. Although not in all groups, serum phosphorus levels increased with increased intake of saturated tallow and unsaturated vegetable oils compared to some groups fed with diets including their lower levels.

In studies conducted out on essential oils, it has been reported that the biological use of calcium increases (Mountzouris et al., 2011) and its fecal excretion decreases (Olgun & Yıldız, 2014) with the addition of essential oil to the diet. In addition, there are also studies reporting that essential oils do not affect plasma minerals (Ali et al., 2007) or reduce their levels (Capkovicova et al., 2014).

Sevim et al. (2020) reported that the highest serum calcium concentration was obtained in the groups fed diets supplemented with orange peel oil (OPO) at 100, 200, 300 and 400 mg/kg, in Japanese quail. The difference between these groups and the groups fed with diet supplemented with OPO at 0 (control) and 50 mg/kg levels was found to be significant ($p<0.01$). Serum phosphorus concentration increased with the addition of OPO to the diet.

Although Bintaş and Özdoğan (2017) found that the addition of boron and zeolite to the diet did not affect serum calcium and phosphorus in old laying hens, Kaya et al. (2019) found that rosa canina seed, which they added to the rations of old laying hens at different levels, significantly increased the blood Ca level (19.08-19.83) compared to the control (12.11) group. Serum Ca and P levels of this study were similar to findings (Ca: 17-25, P: 2.79-5.27) of Kaya et al. (2019) and Nie et al. (2018).

Eren et al. (2003) researched the effect of adding different levels of boron to laying hen diets on serum Ca and P levels at different weeks and reported that increases in serum Ca levels (17.98-25.36) (except for P: 6.75-12.61) were not affected by the treatment, even if it caused changes.

Usayran et al. (2001) investigated the effects of inclusion 4% fat and 4 different levels of non-phytate phosphorus (NPP) to the diet on egg quality in laying hens exposed to high environmental temperatures (35±1 °C) and found that continuous exposure to high environmental temperature reduced serum Ca levels (15.66 vs 17.0) and P (6.91 vs 7.01) along with performance characteristics, but by adding fat improved serum P level (7.01 vs 5.66). Oil supplementation significantly improved P levels, but had no effect on Ca in serum. The trial results showed that there was no significant interaction amongst temperature, NPP supplementation and dietary fat addition for any of the measured criteria.

Abdel-Wareth and Lohakare (2014) examined the addition of the peppermint leaves into the diet at different levels of laying hens during the late laying period and did not observe any significant difference in serum calcium (13.14) and phosphorus (7.16-7.35) amongst treatments and the values remained within the normal range. Also, Atteh and Leeson (1985) investigated the response of laying hens to dietary saturated and unsaturated fatty acids in the presence of varying dietary calcium levels and found that there were no effects on bone Ca and P retentions.

In another study, serum Ca and P levels of hens during the late laying period by supplementing the diet with vitamin D (3000 IU/kg feed) and different levels of P was examined by Çelebi et al. (2005), the highest serum Ca (10.05) level was found in the vitamin D supplemented group; the lowest Ca level (7.90) was observed in the vitamin D-free, P-supplemented (0.45% of feed dry matter) group, which had the highest serum P content (8.42). Likewise, the lowest serum P concentration (4.60) was found in the control and only vitamin D supplemented group (Çelebi et al., 2005). These means related to Ca and P were lower the current research results supplemented with different oils and ratios.

The incompatibility of the results of current research with the findings of other studies may be due to the negative effect of saponification, which occurs as a result of the interaction of oils with different properties and minerals added to rations of animal of different species, breeds and ages on the digestion and absorption of Ca and P. Also, the addition of saturated or unsaturated fats affects the absorption of any of these minerals positively or negatively, it may cause an imbalance by disrupting the ideal ratio between Ca and P resulting in a decrease in bioavailability.

4. Conclusion

Serum Ca and P levels were not affected by the addition of different vegetable oil and animal fat sources at different levels to laying hen diets during the late laying period. On the other hand, the use of high-saturation fats such as tallow in increasing amounts in the diet resulted in a decrease in serum Ca and P levels. However, as the amount of highly unsaturated vegetable oils in the diet increased serum Ca levels also increased, as observed in the group fed with diet containing linseed oil.

Compliance with Ethical Standards

The study was carried out at the Research and Application Farm of the Agricultural Faculty, Atatürk University, before 2006.

Conflict of Interest

The authors declare that they have no conflict of interest.

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