Iranian Journal of Applied Animal Science

Effects of Bovine Bile and Savory Essential Oil Supplemented Diets on Performance, Nutrient Digestibility, Blood Parameters and Antioxidant Status of Broiler Chickens

Research Article

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Received on: 3 Feb 2023
Revised on: 1 Jun 2023
Accepted on: 27 Jun 2023
Online Published on: Sep 2023

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Online version is available on: www.ijas.ir

ABSTRACT

The present experiment was performed to evaluate the effects of bovine bile and savory essential oil in saturated and/or unsaturated fats containing diets on performance, nutrient digestibility, blood parameters and antioxidants of broilers. In this experiment, we used, 480 day-old male broiler of Ross 308 commercial strain, in the form of 2 × 2 × 2 factorial split plots. The results showed that fat sources alone did not have significant effects on chicken performance (P>0.05). Yet, addition of cow bile and savory essential oil to fat containing diets improved feed conversion ratio and increased broiler productive index, by increasing the average daily weight with non-significant effect on daily feed intake (P<0.01). Similar results were observed in the interactions between fat sources, bile, and savory essential oil additives (P<0.05). Ileal nutrient digestibility was higher in canola oil containing diets than in beef tallow containing ones (P<0.01). The use of bile and savory essential oil additives in saturated and/or unsaturated fatty acid containing diets, increased the ileal digestion of nutrients, which was more prominent in beef tallow containing diets than in canola oil containing one. This resulted in interactions between fat sources and additives (P<0.01). The use of beef tallow compared to canola oil increased the blood triglyceride, total cholesterol, high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) levels in chickens (P<0.01). Regarding the antioxidant status of serum, the use of canola oil compared to beef tallow increased the serum level of superoxide dismutase enzyme (P<0.05). Bovine bile additive had no significant effects on the antioxidant status of fat containing diets (P>0.05). Interactions between fat source and type of additive showed that the use of both additives with fat sources improved the serum antioxidant indices of chickens (P<0.05). The general conclusion of the experiment is that in broiler chickens, the use of canola oil compared to beef tallow has positive effects on performance, nutrient digestibility, biochemical and blood immunity parameters, as well as serum antioxidant levels. Additionally, additives in fat containing diets, in particular savory essential oil, were effective on producing health friendly poultry.

KEY WORDS antioxidant status, blood parameters, bovine bile, immunity, performance, savory.

INTRODUCTION

Fats are added to poultry diets for a variety of purposes. High energy content, increased palatability of the diet, reduction of dustiness, supply of essential fatty acids and fat-soluble vitamins are among the reasons for using fat sources in poultry diets (Ali *et al.* 2020). Fats are divided into two groups of saturated and unsaturated fats according to the composition of fatty acids and their nature. Digestibility of oils with higher unsaturated fatty acid content is higher than fats. Oils not only provide essential fatty acids, to chickens but are also good source of energy yet, are more prone to oxidation compared to solid fats (Rodriguez-Sanchez *et al*. 2019). Solid fats are less digestible in poultry (especially at a young age due to digestive limitations) and have less energy compared to oils (Nobakht and Mehman-navaz, 2012). Adding 2% of canola oil to broiler diets compared to fat-free diets improved their performance (Nobakht *et al*. 2022). The use of canola oil up to 6% also improved the performance of broilers (Ebdi and Nobakht, 2017). According to a report, 5% beef tallow diet compared to soybean and canola oils reduced the daily weight gain of chickens (Poorghasemi *et al*. 2015).

Primary bile acids, colic and conodacoxylic acid are a set of water-soluble steroids that are synthesized from cholesterol in hepatic hepatocytes. Primary bile acids conjugate to glycine and / or taurine to form bile salts. Bile salts play a major role in the digestion and absorption of fats and soluble nutrients (Stamp and Jenkins, 2008). Bile salts dissolve better in an acidic environment preventing them from binding to calcium and subsequent precipitation. Bovine bile in fat containing broiler diets improved weight gain and feed conversion ratio (Al-Zawqari *et al*. 2013).

Essential oils are part of the volatile compounds of plant secondary substances that are formed in plants for various reasons. Creating a special smell and aroma to protect plants against invasive agents is one of the causes of the formation of essential oils in plants (Hossein Abadi *et al*. 2022). Due to their nature, plant essential oils have antimicrobial, antifungal, immune and antioxidant properties (Nobakht *et al*. 2022). Essential oil of savory, a well-known medicinal plant with antimicrobial and antioxidant properties, is used in poultry diets for various reasons such as improving performance and immunity of chickens. According to the report of Roostaei Ali Mehr *et al*. (2013), the use of savory essential oil mixed with drinking water of broilers improves the performance and reduces the microbial population of coliform and *Escherichia coli* in the digestive tract of chickens. Another report (Poorghasemi *et al*. 2017), states that the use of two common forms and microcapsules of savory essential oil in the diet of broilers, with insignificant effects on the performance of chickens, delayed the oxidation of chicken breast meat. It has been reported that the use of savory essential oil in the diet of broiler chickens improves the level of immunity and reduces blood lipids and increases the health of the liver of chickens (Zadeh Amiri *et al*. 2013; Sheikhian *et al*. 2018). Fats, being low energy source, have shown to improve performance and immunity increasing the efficiency of diets in poultry. Nevertheless, low digestibility and sensitivity to oxidation of fats limit their utilization. In the present experiment, we investigated the effects of bovine bile and savory essential oil supplemented fat containing diets on efficiency, nutrient digestibility, immunity and antioxidants status and biochemical parameters of chicken blood.

MATERIALS AND METHODS

Four hundred eighty one-day-old Ross 308 male chicks were used in this experiment. The design of experiment was 2 × 2 × 2 factorial split plots, with diets supplemented with either beef tallow or canola oil, including two levels of bile (zero and 0.5%) and two levels of savory essential oil (zero and 0.04%). We examined two types and two levels of fat sources in a completely randomized design with 8 treatments and 5 replications as sub-plots. The fat sources used in the diet included 4% of beef tallow and canola oil. The experiment was performed in three periods including starter (1 to 10 days), grower (11 to 24 days) and finisher (25 to 42 days). Diets were formulated for broiler chickens according to the nutrient requirements listed in Ross 308 (2014) catalog using UFFDA software. All chickens had free access to water and food during experiment period. Bovine bile was obtained from a semi-industrial cattle slaughterhouse. Beef tallow was acquired from a semi-industrial slaughterhouse unit, melted and the impurities were separated then transferred to the poultry farm. Other ingredients were grinded and gradually added to the diets in the mixer to be mixed with the rest of the ration items. Canola oil, as a source of unsaturated fat, was purchased and like beef tallow was added to other milled food ingredients to experimental diets. Completely homogeneous dehydrated bile was used in the required amount in diets. Savory essential oil (purchased from Barij Essential Oil Pharmaceutical Company, Kashan, Iran) was sprayed in the required amount on diets three days before ration feeding and then completely mixed with the entire contents. The ration ingredients used as well as the chemical composition of the diets are listed in Table 1.

After categorizing the animals, the chickens were randomly weighed in groups and placed in experimental cages. In the first 3 days, a permanent lightning program was applied to the chicks, and after than one hour dark and 23 hours lightning program was applied to the chicks. The temperature of the chickens was initially 37 ˚C, which was reduced by 2 degrees per week, and at the end of the fifth week until the end of the experimental period was kept in the range of 18 to 24 ˚C. The humidity of the chickens rearing hall in the beginning was in the range of 55 to 60 percent, which in the following weeks of rearing reached 65 to 70 percent. Weight and feed intake of chickens in the experimental units were measured at the end of experimental period with a scale (accuracy of 0.01 g).

Table 1 Diet ingredients and nutrient compositions of diets used to broilers feeding in different period of the experiment

Feeds ingredients	**Starter (1-10 days)**		**Grower (11-24 days)**		**Finisher (25-42 days)**	
Fat source	Canola oil	Beef tallow	Canola oil	Beef tallow	Canola oil	Beef tallow
Corn	48.00	48.00	54.03	54.52	56.00	55.46
Soybean meal (42% CP)	44.99	44.12	37.90	38.02	36.65	38.30
Canola oil	4.00	0	4.00	0	4.00	0
Beef tallow	0	4.00	0	4.00	0	3.43
Dicalcium phosphate	2.38	2.26	2.18	2.34	0	0
Bone meal	0	0	0.29	0.31	1.87	1.20
Oyster shell	0.29	0.29	0.29	0.31	0.33	0.37
Salt	0.46	0.46	0.47	0.50	0.33	0.37
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.37	0.37	0.34	0.36	0.38	0.42
Calculated composition						
Metabolisable energy (kcal/kg)	3100	3100	3150	3150	3200	3200
Crude protein (%)	23.00	23.00	21.00	21.00	20.00	20.00
Calcium (%)	0.70	0.70	0.75	0.75	0.73	0.73
Available phosphorus (%)	0.35	0.35	0.33	0.33	0.34	0.34
Sodium (%)	0.14	0.14	0.13	0.13	0.13	0.13
Lysine (%)	1.06	1.06	1.00	1.00	0.95	0.95
Methionine + cysteine (%)	0.98	0.98	0.89	0.89	0.87	0.87
Methionine (%)	0.64	0.64	0.55	0.55	0.54	0.54

[1] Vitamin premix per kg of diet: vitamin A (retinol): 2.7 mg; vitamin D_3 (calciferol): 0.05 mg; vitamin E (tocopherol acetate): 18 mg; vitamin K_3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg.

[2] Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu ($CuSO_4.5H_2O$): 10 mg; I (K_1, 58% I): 1 mg and Se ($NaSeO_3$, 45.56% Se): 0.2 mg.

Flock survival percentage was calculated according to the percentage of mortality of experimental groups at the end of the breeding period. To calculate the production index in chickens, the survival rate of chickens was multiplied by the live weight of the chickens at the end of the rearing period, and then the weight at the time of slaughter was multiplied by the feed conversion ratio and then the deducted number was divided by its denominator. To determine the ileal digestibility of dietary nutrients, at the end of the starter and growth stages, the ileal contents of chickens were sampled and analyzed by slaughter methods to determine the digestibility of dry matter, protein, and energy of diets (Kluth *et al*. 2005).

To measure ileal digestibility and energy, we used ileum contents' sampling method with chromium oxide as the marker. Sampling was done in the starter and growing periods up to 2 g and the samples were kept at -20 ˚C until further analysis. For chemical analysis of ileum contents, the samples were dried at 60 ˚C, grounded and the digestibility of dry matter, crude protein, and crude fiber as well as the types of energy content of ileum content were calculated (Sun *et al*. 2020). According to the animal requirements, the necessary needs were met (Yaghobfar, 2016). For blood biochemical parameters analysis, blood samples were taken from the jugular vein of two chickens (after 6 hours of starvation) from each experimental unit and in the laboratory using authorized diagnostic kits to determine cholesterol, triglyceride, albumin, uric acid, HDL, and (LDL). To determine the level of immunity of chickens' blood, the percentage of heterophiles, lymphocytes and the ratio of heterophils to lymphocytes were measured in the hematology laboratory (Hamidi *et al*. 2022). To assess the antioxidant status of chickens' blood, the blood glutathione peroxidase levels, superoxide dismutase, catalase and thiobarbituric acid were evaluated (Hosseini Vashan *et al*. 2012).

Differences between groups were analyzed with analysis of variance (ANOVA) by using the statistical software SAS (2004). Significant means were subjected to a multiple comparison test (Tukey) at α= 0.05 level.

RESULTS AND DISCUSSION

The effects of using different fats and additives as well as the interactions between those are described in Table 2. Fat type had no significant effects on the performance of chickens in the whole experimental period (P>0.05). Yet, additive containing diets (bovine bile and savory essential oil) with non significant effect on daily feed intake, improved feed conversion ratio and increased broiler production index by improving the amount of daily weight gain (P<0.01).

Similar results were observed in the interactions between different fat sources and additives (bile and savory essential oil) (P<0.05).

Table 2 Effects of fats, savory and bile on performance of broilers (42 day)

Performance	Feed intake (g/chick/d)	Weight gain (g/chick/d)	Feed conversion ratio	Livability	Production index
Fat source					
Canola	101.91	50.16	1.90	95.42	235.61
Beef tallow	101.77	49.54	1.92	95.42	231.79
SEM	0.15	0.29	0.01	1.20	5.00
P-value	0.516	0.141	0.429	1	0.59
Bile (Fat)					
0 (Canola)	101.97	48.75[b]	1.96[a]	95.00	214.19[b]
0.5 (canola)	101.84	51.57[a]	1.84[b]	95.83	257.03[a]
0 (Tallow)	102.14	48.45[b]	1.97[a]	94.17	209.77[b]
0.5 (Tallow)	101.40	50.63[a]	1.87[b]	96.67	253.80[a]
SEM	0.21	0.41	0.02	1.69	7.07
P-value	0.0573	0.0001	0.0001	0.5516	0.0001
Savory × (fat)					
0 (Canola)	102.06	49.20[b]	1.95[a]	95.00	226.92[ab]
0.04 (Canola)	101.76	51.12[a]	1.86[b]	95.83	244.30[a]
0 (Tallow)	101.48	48.81[b]	1.94[a]	95.00	220.42[b]
0.04 (Tallow)	102.06	50.28[b]	1.90b	95.83	243.15[a]
SEM	0.21	0.41	0.02	1.69	7.07
P-value	0.114	0.0009	0.002	0.886	0.0262
Bile × Savory × Fat					
0 × 0 × Canola	101.91	48.02[cd]	1.99[ab]	95.00	206.52[bc]
0 × 0.04 × Canola	102.06	49.47[bcd]	1.93[abc]	65.00	221.86[abc]
0.5 × 0 × Canola	102.21	50.37[abc]	1.90[abc]	95.00	247.32[ab]
0.5 × 0.04 × Canola	101.47	52.77[a]	1.79[d]	96.67	266.73[a]
0 × 0 × Tallow	101.98	47.50[d]	2.00[a]	93.33	193.73[c]
0 × 0.04 × Tallow	102.30	49.40[bcd]	1.94[abc]	95.00	226.32[abc]
0.5 × 0 × Tallow	100.99	50.12[bcd]	1.88[bc]	96.67	247.32[ab]
0.5 × 0.04 × Tallow	101.82	51.15[abc]	1.85[cd]	96.67	260.27[a]
SEM	0.30	0.58	0.02	2.39	1.00
P-value	0.2464	0.0423	0.411	0.8863	0.0119

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The effects of various fats and additives on ileal nutrient digestibility in starter and grower periods are given in Table 3. The use of different sources of fat and the type of additive have had significant effects on the digestibility of nutrients in diets in the early and growing periods (P<0.01). Canola oil compared to beef tallow in the starter period improved digestion of dry matter and apparent metabolizable energy. In the growing period, the effect of canola oil on the digestibility of dietary nutrients was greater than the starter period (P<0.01).

The effects of fat source and type of additive on biochemical parameters of broiler blood are shown in Table 4. The use of different sources of fat alone or with the type of additive had significant effects on some of blood biochemical parameters (P<0.01).

Canola oil significantly reduced serum total cholesterol, triglyceride, LDL and VLDL concentrations compared to beef tallow. The use of additives in fat containing diets accelerated the reduction of blood lipid levels, meaning that the effect of additives in reducing the concentration of blood lipid was greater than the effects of separate additives. Experimental diets had no significant effects on blood LDL, uric acid, phosphorus, total protein, and albumin levels in chickens (P>0.05).

The effects of using different sources of fat and type of additive on immunity and antioxidant status of chickens' blood are shown in Table 5. The use of different sources of fat with additives had significant effects on the level of immunity and antioxidant status of chickens' blood (P<0.01).

Table 3 Effects of fats, savory and bile on the digestibility of some nutrients in broiler diets in starter and grower periods

Item	Starter period					Grower period				
	Dry matter	Crude protein	Crude fiber	AME	AMEn	Dry matter	Crude protein	Crude fiber	AME	AMEn
Fat source										
Canola	71.73a	62.41	75.67	2823.70^{a}	2783.5	74.95^{a}	63.56^{a}	77.15^{a}	2907.75^{a}	2841.15
Beef tallow	70.21^{b}	61.74	74.65	2757.80^{b}	2579.5	72.13^{b}	61.93^{b}	75.49^{b}	2853.05^{b}	2824.5
SEM	0.27	0.26	0.38	13.25	91.62	0.20	0.26	0.26	10.24	12.36
P-value	0.0003	0.0778	0.0641	0.0013	0.1252	0.0001	0.0001	0.0001	0.0007	0.348
Bile × Fat										
0 (Canola)	71.35^{a}	61.86	74.89	2758.2^{bc}	2677.10	74.49^{a}	63.31^{ab}	75.67^{b}	2841.40^{c}	2758.50^{b}
0.5 (canola)	72.11^{a}	62.96	76.46	2889.2^{a}	2889.90	75.42^{a}	63.81^{a}	78.63^{a}	2974.10^{a}	2923.80^{a}
0 (Tallow)	69.07^{b}	61.79	73.59	2705.4^{c}	2384.41	71.15^{c}	61.71^{c}	73.43^{c}	2791.90^{c}	2780.90^{b}
0.5 (Tallow)	71.37^{a}	61.69	75.70	2810.2^{ab}	2774.60	73.12^{ab}	62.16^{bc}	77.56^{b}	2914.20^{b}	2868.10^{a}
SEM	0.38	0.37	0.54	18.73	129.58	0.28	0.37	0.37	14.48	17.48
P-value	0.0003	0.1193	0.0062	0.0001	0.0467	0.0001	0.0443	0.0001	0.0001	0.0001
Savory × (Fat)										
0 (Canola)	71.71^{a}	62.63	75.27	2769.4^{b}	2726.6	74.58^{a}	64.32^{a}	76.69^{b}	2870.0^{b}	2809.8^{ab}
0.04 (Canola)	71.75^{a}	62.19	76.08	2878.0^{a}	2840.4	75.33^{a}	62.80^{b}	77.60^{a}	2945.5^{a}	2872.5^{a}
0 (Tallow)	69.71^{b}	61.33	74.51	2750.9^{b}	2433.41	72.26^{b}	61.51^{b}	74.27^{b}	2845.7^{b}	2804.8^{b}
0.04 (Tallow)	70.72^{b}	62.15	74.79	2764.7^{b}	2725.6	72.01^{b}	62.35^{b}	76.72^{a}	2860.4^{b}	2844.2^{b}
SEM	0.38	0.37	0.54	18.73	129.57	0.28	0.37	0.37	14.48	17.48
P-value	0.0185	0.2143	0.5404	0.0011	0.2463	0.0153	0.0085	0.0001	0.0029	0.0192
Bile × Savory × Fat										
0 × 0× Canola	71.38^{ab}	62.24	75.12	2689.0^{d}	2624.2	73.64^{bc}	65.20^{a}	75.55^{cd}	2775.80^{b}	2744.0^{d}
0 × 0.04 × Canola	71.32^{ab}	61.73	74.67	2827.4^{ab}	2730.0	75.33^{ab}	61.41^{c}	75.78^{bcd}	2907.0^{a}	2773.0^{cd}
0.5 × 0 × Canola	72.36^{a}	63.02	75.43	2849.8^{a}	2829.0	75.52^{a}	63.43^{abc}	77.83^{abc}	2964.2^{a}	2875.6^{abc}
0.5 × 0.04 × Canola	72.19^{a}	62.90	77.49	2928.6^{a}	2950.8	75.33^{ab}	64.18^{ab}	79.42^{a}	2984.0^{a}	2972.0a
0 × 0× Tallow	68.86^{c}	61.73	73.66	2691.0^{cd}	2079.4	71.16^{d}	61.57^{c}	71.51^{e}	2781.4^{b}	2775.2^{cd}
0 × 0.04 × Tallow	69.27^{bc}	61.85	73.52	2719.8^{bcd}	2689.4	71.13^{d}	61.84^{c}	75.34^{d}	2802.4^{b}	2786.6^{cd}
0.5 × 0 × Tallow	70.56a^{bc}	60.93	75.34	2810.8a^{bcd}	2787.4	73.35^{c}	61.45^{abc}	77.03^{d}	2910.0^{a}	2834.4^{bcd}
0.5 × 0.04 × Tallow	72.17^{abc}	62.45	76.05	2809.6	2761.8	72.88^{cd}	62.87^{abc}	78.09^{ab}	2918.4^{a}	2901.8^{ab}
SEM	0.53	0.52	0.76	26.49	183.24	0.40	0.52	0.53	20.48	24.72
P-value	0.0002	0.3440	0.0199	0.0001	0.0237	0.0001	0.0004	0.0230	0.0346	0.0001

AME: apparent metabolizable energy and AMEn: apparent metabolizable energy corrected for nitrogen.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Compared to beef tallow, canola oil increased the percentage of heterophils and the ratio of heterophils to lymphocytes and decreased the percentage of heterophils (P<0.01). The use of additives with fat sources and the interaction effects of additives with fat sources increased the percentage of heterophils to lymphocytes and also the ratio of heterophils to lymphocytes and decreased the percentage of heterophils compared to using only fat sources, which improved the level of immunity. This was more prominent when using additives with beef tallow. Regarding the antioxidant status of blood, the use of canola oil compared to beef tallow increased the level of glutathione peroxidase in chickens' blood (P<0.05). Addition of bovine bile to fat containing diets had no significant effects on the antioxidant status of chickens' blood (P>0.05).

Savory essential oil significantly increased blood glutathione peroxidase, superoxide dismutase and catalase levels while decreasing thiobarbiotic levels in chickens (P<0.01). The addition of bovine bile and savory essential oil to fat sources, especially in combination improved the antioxidant status of the blood (P<0.05).

Experimental diets were identical in terms of energy, protein, vitamins and minerals but formulated with fats from different sources. Fat source, according to our results, did not affect broiler performance.

The lack of improvement in performance due to the addition of different sources of fat can be related to the energy balance of the diets.

Table 4 Effects of fats, savory and bile on blood biochemical parameters of broilers

Item	Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (g/dL)	LDL (mg/dL)	Uric Acid (g/dL)	Phop (g/dL)	TP (g/dL)	Albu (g/dL)
Fat source									
Canola	98.20[b]	70.95[b]	43.45[b]	14.19[b]	44.56	3.82	7.12	4.63	1.46
Beef tallow	130.00[a]	93.90[a]	57.70[a]	18.78[a]	53.52	4.59	6.50	3.85	1.39
SEM	3.60	2.31	2.65	0.46	4.25	0.34	0.21	0.30	0.07
P-value	0.0001	0.0001	0.0006	0.0001	0.0788	0.1188	0.899	0.0800	0.5275
Bile × Fat									
0 (Canola)	102.60[bc]	77.10[c]	454.70[ab]	15.42[bc]	41.48	3.79	6.97	4.62	1.44
0.5 (canola)	93.80[c]	64.80[c]	41.20[b]	12.96[c]	39.64	3.84	7.27	4.63	1.48
0 (Tallow)	139.50[a]	98.50[a]	57.60[a]	19.70[a]	62.20	4.91	6.72	3.81	1.37
0.5 (Tallow)	120.50[b]	89.30[ab]	57.80[a]	17.86[ab]	44.84	4.27	6.28	3.89	1.42
SEM	5.09	3.27	3.75	0.65	6.01	0.48	0.29	0.43	0.1
P-value	0.0235	0.0087	0.0200	0.0087	0.3066	0.6475	0.4383	0.9912	0.8994
Savory × (Fat)									
0 (Canola)	100.50[b]	72.40[b]	41.40[b]	14.48[b]	44.62	3.44	6.91	4.60	1.48
0.04 (Canola)	95.90[b]	69.50[b]	45.50[b]	13.90[b]	36.50	4.19	7.33	4.65	1.44
0 (Tallow)	139.50[a]	97.50[a]	61.20[a]	19.50[a]	58.80	4.53	6.48	3.82	1.37
0.04 (Tallow)	120.50[a]	90.30[a]	54.20[ab]	18.06[a]	48.24	4.65	6.52	3.88	1.42
SEM	5.09	3.27	3.75	0.65	6.01	0.48	0.29	0.43	0.1
P-value	0.0364	0.0259	0.0324	0.0259	0.3066	0.5260	0.5930	0.9917	0.8994
Bile × Savory × Fat									
0 × 0× Canola	106.20[bc]	75.00[bcd]	42.60[ab]	15.00	48.60	3.02	6.72	4.52	1.46
0 × 0.04 × Canola	99.00[bc]	79.20[abcd]	48.80[ab]	15.84	34.36	4.56	7.22	4.72	1.42
0.5 × 0 × Canola	94.80[c]	69.80[cd]	40.20b	13.96	40.64	3.86	7.10	4.68	1.49
0.5 × 0.04 × Canola	92.80c	59.80[d]	42.20[ab]	11.96	38.64	3.82	7.44	4.58	1.46
0 × 0× Tallow	150.20[a]	99.20[a]	63.00[a]	19.84	67.36	4.70	6.48	3.64	1.34
0 × 0.04 × Tallow	128.80[ab]	97.80[a]	52.20[ab]	19.56	57.04	5.12	6.96	3.98	1.40
0.5 × 0 × Tallow	128.80[ab]	95.80[ab]	59.40[ab]	19.16	50.24	4.36	6.48	4.00	1.40
0.5 × 0.04 × Tallow	112.20[c]	82.80[abc]	56.20[ab]	19.56	39.44	4.18	6.08	3.78	1.44
SEM	7.20	4.62	5.31	0.92	8.50	0.68	0.40	0.61	0.14
P-value	0.0001	0.0001	0.0309	0.0001	0.7732	0.4744	05565	0.8722	0.9950

Chol: cholesterol; TG: triglyceride; HDL: high density lipoprotein; VLDL: very low density lipoprotein; LDL: low density lipoprotein; Phop: Phosphoric; TP: total protein and Albu: albumin.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Our results did not agree with previous studies reporting the positive effects of adding unsaturated fats to the broiler s' diet on performance (Nobakht *et al*. 2022; Poorghasemi *et al*. 2015; Ebdi and Nobakht, 2017).

The type and amount of fats as well as the composition of diets and the health status of broiler s could be the source of this variation (Poorghasemi *et al*. 2013). The inconsistency of the results of this experiment with the previous results can be related to the source of fat, other components of the diet, the level of health of the broilers and the way of preparing and feeding the diets. Though bovine bile and savory essential oil did not affect the feed intake but could significantly improve broiler health possibly via their antioxidant and antimicrobial properties, alter intestinal cell morphology and improve digestion and absorption. Improved performance of chickens after the consumption of bovine bile supplemented diet has been assumed to be associated with the fat emulsifying effect of bile and better activity of bile salts to in the acidic gastrointestinal tract environment (Al-Zawqari *et al*. 2013). The antioxidant, antimicrobial, digestion, and nutrient uptake stimulation properties of different savory derivatives have been reported to be linked with improved performance of the broilers (Roostaei Ali Mehr *et al*. 2013). Positive effect of savory essential oil on the performance of broilers has not been previously reported (Zadeh Amiri *et al*. 2013).

Table 5 Effects of fats, savory and bile on blood immunity[1] and antioxidant status of broilers

Item	Heterophile (%)	Lymphocyte (%)	H/L	Glutathione peroxides (g/dL)	SOD (mg/dL)	CAT (g/dL)	TBARS (g/dL)
Fat source							
Canola	59.00^{a}	37.90^{b}	1.62^{a}	20.15^{a}	1072.80	170	331.4
Beef tallow	51.70^{b}	48.30^{a}	1.12^{b}	18.95^{b}	940.75	153.45	313.1
SEM	1.58	1.34	0.06	0.50	43.70	7.63	13.17
P-value	0.0025	0.0001	0.0001	0.1006	0.0404	0.1349	0.333
Bile × Fat							
0 (Canola)	60.30^{a}	39.00^{b}	1.65^{a}	19.80	1059.60	168.50	340.90
0.5 (canola)	57.70^{ab}	36.80^{b}	1.59^{a}	20.50	1086.00	171.50	321.90
0 (Tallow)	49.70^{b}	48.90^{a}	1.07^{b}	18.50	921.00	148.40	323.400
0.5 (Tallow)	53.70^{ab}	47.70^{a}	1.17^{b}	19.40	960.00	158.50	302.800
SEM	2.23	1.89	0.08	0.71	61.80	10.79	18.62
P-value	0.3306	0.0438	0.0493	0.5313	0.8632	0.7893	0.5731
Savory × (Fat)							
0 (Canola)	53.00^{bc}	41.60^{b}	1.32^{b}	15.10^{b}	889.60^{b}	103.00^{c}	413.80^{a}
0.04 (Canola)	65.00^{a}	34.20^{c}	1.92^{a}	25.20^{a}	1259.00^{a}	237.00^{a}	249.00^{b}
0 (Tallow)	46.90^{c}	53.90^{a}	0.89^{c}	14.20^{b}	670.50^{b}	111.40^{c}	376.20^{a}
0.04 (Tallow)	56.50^{ab}	47.70^{b}	1.35^{b}	23.70^{a}	1211.00^{a}	195.50^{b}	250.00^{b}
SEM	2.23^{ab}	1.89	0.08	0.71	61.80	10.79	18.62
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Bile × Savory × Fat							
0 × 0× Canola	53.80^{abc}	49.00^{abc}	1.21^{bcd}	14.40^{b}	834.2^{bc}	99.0^{b}	430.8^{a}
0 × 0.04 × Canola	66.80^{a}	32.00^{d}	2.10^{a}	25.20^{a}	1285.0^{a}	238.0^{a}	251.0^{b}
0.5 × 0 × Canola	52.20^{bc}	37.20^{cd}	1.43^{bc}	15.80^{b}	939.0^{abc}	107.0^{b}	396.8^{a}
0.5 × 0.04 × Canola	63.20^{ab}	36.40^{cd}	1.75^{ab}	25.20^{a}	1233.0^{ab}	236.0^{a}	247.0^{b}
0 × 0× Tallow	44.20^{c}	54.60^{a}	0.82^{d}	13.80^{b}	608.0^{c}	101.0^{b}	395.8^{a}
0 × 0.04 × Tallow	55.20^{abc}	43.20^{abcd}	1.32^{bcd}	23.20^{a}	1234.0^{ab}	195.0^{a}	251.0^{a}
0.5 × 0 × Tallow	49.60^{bc}	53.20^{ab}	0.96^{cd}	14.60^{b}	733.0^{c}	121.0^{b}	356.6^{ab}
0.5 × 0.04 × Tallow	57.80^{abc}	42.20^{bcd}	1.38^{bc}	24.20^{a}	1188.0^{ab}	196.0^{a}	249.0^{b}
SEM	3.15	2.67	.12	1.00	87.40	15.26	26.33
P-value	0.0004	0.0001	0.0001	0.0423	0.0325	0.0321	0.0124

H/L: heterophile/lymphocyte; SOD: super oxide dismutase; CAT: catalase and TBARS: thiobabitic acid reactive substances.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The observation results can be related to the synergy created between the effective ingredients in the savory essential oil and fat sources, which has improved the level of health and better absorption of nutrients by the broilers.

Increasing the digestibility of dietary nutrients using canola oil compared to beef tallow may be due to the better bio-supply of energy released by easy and better digestion of canola oil compared to beef tallow. Oils with higher unsaturated fatty acids than solid fats (offer better digestibility and energy potential compared to saturated fatty acids (Poorghasemi *et al.* 2015). Increased essential nutrient digestion efficiency during the growth period compared to the starter period can be related to the acquisition of more digestive abilities of chickens with age (Tancharoenrat *et al.* 2013).

The use of bovine bile along with fat sources compared to control group in the starter and grower periods, improved the digestion of dry matter and essential nutrients which was more prominent in beef tallow fed chickens than canola oil fed ones.

The observed performance improvement can be partly due to weight gain and improvement in digestion and bioavailability of most of the nutrients contained in the diet, especially protein and amino acids. It also includes improvements in the crude protein digestibility of diets during the growing period. Regarding the greater effect of added bile on the digestibility of nutrients in diets containing beef tallow compared to canola oil, it is inferred that this action was related to the digestive constraints in beef tallow compared to canola oil.

By creating fat emulsion and breaking them into small components, bile provides the basis for the action of the lipase enzyme, and by increasing the absorption and better bioavailability of energy, it provides the basis for improving performance. Beef tallow is a source of saturated fatty acids with digestive limitations for a variety of reasons. The role of bile as an improver may be related to better digestion of beef tallow with different mechanisms and increased energy production and improved digestibility of nutrients in diets.

Although the addition of savory essential oil to fat containing diets compared to control group, improved the digestibility of nutrients in the starter and growing periods, yet was less effective compared to bovine bile. Bile as an emulsifier in fat-containing diets plays a key role in crushing fat particles, increasing the level of lipase enzyme effect, improving digestion and release of their energy content, while savory essential oil mainly has antioxidant properties. Most significant effect on the ileum nutrient digestibility was observed with the combined use of both additives in canola oil and / or beef tallow containing diets. The better performance observed in the combined use of bovine bile and savory essential oil can be related to the proper synergy between an emulsifier and an antioxidant, which together have improved the use of fat sources. This can be due to the synergy between these two additives, which in all cases compared to control and/or additive alone improved the digestibility of nutrients. Increased hyperlipidemia (cholesterol, triglyceride, HDL, and LDL) in diets containing beef tallow compared to canola oil can be due to the lack of cholesterol in plant vegetable fat sources and its lower digestibility and energy production.

Baighi and Nobakht (2017) reported that 4% saturated and/or unsaturated fat diet has insignificant effects on blood lipid concentrations. The difference can be due to the sources of fat used, the health status of the chickens as well as other components of the diet.

Reducing serum levels of blood lipids in diets containing fat sources by adding bovine bile, indicates the positive effects of bovine bile on emulsification, increasing the effect of lipase enzyme and subsequently improving digestion and absorption of lipid sources and their beneficial use (Al-Zawqari *et al*. 2013).

The addition of savory essential oil to fat containing diets altered the serum level of fat constitutes yet in comparison to bovine bile was statistically insignificant. The difference in the effects of these two additives on the level of blood lipids can be due to their mode of action. Bile exerts its effect mainly by emulsifying fats and increasing the effect of lipase enzyme thus improving digestion and high energy production by fat subsequently reducing blood lipid levels.

Savory derivatives mainly protect fats from oxidation through its antioxidant properties. Additives showed the greatest effect in reducing the serum lipid levels when used in combination of both fat sources (Sheikhian *et al*. 2018).

Increasing the percentage of heterophils and the ratio of heterophils to lymphocytes and decreasing the percentage of heterophiles in the blood would improve immunity in birds (Nazifi, 1997). Canola oil has more unsaturated fatty acids and essential fatty acids than beef tallow. Furthermore, role of essential fatty acids in increasing the level of immunity has been repetitively demonstrated. In accordance, canola oil fed birds in our study had better immune system. While bovine bile supplemented fat containing diets did not have significant effects on improving the immune status compared to control. Savory essential oil group compared to control had higher percentage of heterophils as well as heterophils to lymphocytes ratio with lower percentage of lymphocytes. Savory essential oil, containing immunogenic compounds such as vitamins, phenolics and effective secondary substances, have shown to stimulate the growth of immune cells increasing the immunity of chickens (Dehghani *et al*. 2018). In studying the interactions between fat sources and additives on the immune system, the best result was observed with the combined use of these two additives with fat sources, which was due to the phenomenon of overlap between the two additives in proliferating immune cells and improving the immune system (Zadeh Amiri *et al*. 2013; Poorghasemi *et al*. 2017).

Increased blood levels of superoxide dismutase in the canola oil containing diet indicate greater antioxidant effects compared to beef tallow, which may be due to the presence of certain compounds such as more essential fatty acids as well as PUFA (omega-3 and omega 6). Addition of savory essential oil to diets containing different fat sources increased the levels of glutathione peroxidase, superoxide dismutase, catalase and decreased the level of thiobarbiotics. Although the addition of bovine bile to diets containing fat sources alone did not have any positive effects on the levels of antioxidant enzymes in the serum of chickens, the combined use of these two additives interaction between fat sources and type of additive, effectively improved the antioxidants enzymes levels compared to control or individual use of each additive (Sheikhian *et al*. 2018).

CONCLUSION

Can be concluded that in broilers, the use of 4% of saturated and unsaturated fat sources along with bovine bile and savory essential oil have positive effects on performance, nutrient digestibility, reduction of blood lipids, immunity and antioxidant status of chickens.

ACKNOWLEDGEMENT

The authors thank all the teams who worked on the experiments and provided results during this study.

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