RESEARCH PAPER

POULTRY STUDIES

Different Concentrations of High Fructose Corn Syrup in Broiler Diet Cause Different Effects on Selected Hematological Parameters

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Abstract

This study was aimed to investigate how the consumption of high fructose corn syrup (HFCS) affects blood parameters of broilers. Total 120 chickens were divided into three groups including 4 subgroups in each with free access to food and water for 42 days. The control group received no additional treatment, while the second and third groups were fed diets containing 5% or 10% HFCS, respectively. On the last day, broilers were euthanized, and 5 mL blood samples were collected for hematological analyses. Results indicated no significant differences in red blood cell (RBC) or hemoglobin (HGB) levels between the control and 5% or 10% HFCS-treated groups. However, 10% HFCS treatment significantly increased packed cell volume (PCV) and mean corpuscular volume (MCV), while 5%-HFCS increased mean corpuscular hemoglobin (MCH) and decreased mean corpuscular hemoglobin concentration (MCHC) compared to the control group. Total leukocyte counts (TLC) and monocyte values remained unaffected by HFCS treatments. Notably, 5% HFCS treatment increased basophil, heterophil, and heterophil/lymphocyte ratio while decreasing lymphocyte. Conversely, 10% HFCS treatment reduced eosinophil and heterophil, increasing lymphocyte counts. In conclusion, our study suggests that dietary fructose intake can modify certain hematological parameters, potentially serving as early indicators of future systemic or metabolic issues.

Introduction

Cells necessitate a continuous influx of energy to maintain the essential biological processes that sustain their vitality. This energy is sourced from the chemical bond energy present in food molecules, which consequently act as cells' energy fuel (Alberts 2002). Carbohydrates serve as a vital energy source, play a role in regulating blood glucose and insulin metabolism, contribute to cholesterol and triglyceride metabolism, and are involved in fermentation. The process of carbohydrate breakdown into monosaccharides is initiated in the digestive tract (Goñí et al., 2007; Şen and Başalan 2017). Monosaccharides are also called carbohydrate sweeteners and highly desired due to their sweetening properties, as they elevate the flavor and overall enjoyment of a wide range of foods (Parker et al., 2010). Fructose monosaccharide is one of the three primary monosaccharides, along with glucose and galactose, and is the naturally sweetest among them (Skoog and Bharucha 2004). It is found in nearly all processed foods, fruits, vegetables, grains, and cereals. In addition, fructose is extensively utilized as a sweetener alternative to glucose or sucrose in the food industry and has become a significant component of contemporary diets (Okon et al., 2019). Following the development of high fructose corn syrup (HFCS) as a cheaper alternative to natural sugars, the use of HFCS in foods and beverages industry has gradually increased and reached approximately 40 percent of additive sweeteners in US (Parker et al., 2010). Therefore, fructose is employed in the production of desserts, condiments, and carbohydrate beverages. This situation has led to serve fructose as a big energy source for people of all ages worldwide (Fedewa and Rao 2014). However, as reviewed by Okon et al. (2019), the excessive consumption of refined carbohydrates in food and beverages elevates the risk of conditions such as dyslipidemia, obesity, insulin resistance, and heart disease. Furthermore, it is well known that the measurement of hematological parameters or indices has proven to be a valuable method for diagnosing diseases and evaluating the health of both animals and humans. This is because blood frequently provides specific indications of ongoing physiological processes in the body, assisting in the diagnosis and assessment of health status (Theml et al., 2004; Kabakci 2022; Kabakci and Kara 2023).

Recently, there has been a growing utilization of fruit-derived by-products in poultry farming. However, the immediate impact of fructose on the metabolism of young chickens remains unclear, as these by-products likely contain fructose. This mean that it is imperative to conduct research to assess the fructose consumption among poultry (Goñí et al., 2007; Ebrahimi et al., 2013; Akhlaghi et al., 2014). Therefore, this study was conducted to evaluate the effects of dietary different concentrations of high fructose corn syrup (HFCS) consumption on selected hematological parameters in broilers. We also assessed the heterophil to lymphocyte ratio (H/L) as stress and well-being parameter in poultry which is still remains justified (Scanes 2016).

Material and Methods

The experimental design of this study was approved by Kirikkale University Local Ethical Committee by the number of 2020-06/06. Following a power analysis, the experiment was carried out with one hundred twenty 0-day-old Ross 308 broiler chickens and lasted 42 days. Broiler chickens were divided into 3 groups with 4 subgroups, each 10 chickens were housed in an area of 1 m^2 on the ground as described in Ross Manuel User (Aviagen 2014). The groups consisted of as follows; group I fed with an HFCS-free diet (control), group II fed with diet containing 5% HFCS (low) and group III fed with diet containing 10% HFCS (high). The nutritional needs of the broiler chickens were determined according to (NRC 1994). The diets were prepared as isocaloric and isonitrogenic. Diets and water are recommended as *ad libitum* in compliance with the Ross broiler breeder's handbook (Aviagen 2014). The HFCS purchased from Sunar Mısır (Sunar Mısır Entegre Sanayi ve Ticaret A.Ş. Seyhan/Adana) and contained 42% fructose. The broiler chickens were housed in a heated environment with electric heaters and illuminated with fluorescent lights for 24 hours. Lighting periods were performed as previously described by Ouchi et al. (2023). The environmental temperature was initially set at 35 ºC and then gradually reduced as recommended in the Ross broiler breeder's handbook (Aviagen 2014).
At the end of the experimental days, animals were killed by cervical dislocation and blood samples (5mL) were collected bleeding jugular vein into test tubes with anticoagulant (K_3EDTA) for hematological analysis. Total leukocyte counts (TLC) and red blood cells (RBC) were counted using hemocytometer methods with Turk solution and Hayem solution (Tamzil et al., 2016), respectively. Hematocrit (PCV) and hemoglobin (HGB) concentration were determined by microhematocrit methods and cyanmethemoglobin methods (Campbell and Ellis 2007), respectively. Then, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as red blood cell indexes were calculated from the values of RBC, PCV, and HGB. Percentage of leukocyte subtypes were determined by manually counting total 100 cells: Basophil, Eosinophil, Heterophil, Lymphocyte, and Monocyte from the blood smear under a light microscope. Afterwards heterophil/lymphocyte (H/L) rate was calculated for each sample (Gross 1983).
Descriptive and statistical analysis of the data were performed by SPSS 20.0 with one-way ANOVA following the Normality test. The significancy of difference between the groups were evaluated by post-hoc Tukey test. $P<0.05$ was considered as statistically significant.

Results

Effects of %5 or 10% levels of dietary HFCS intake on selected hematological parameters in broiler were presented in Table 1. Although the application of 5% dietary HFCS intake decreased the number of erythrocyte and high dietary HFCS intake increased the number of erythrocytes, these were not statistically different from control group ($P>0.05$). However, RBC was significantly higher in 10%-HFCS group than that of 5%-HFCS group ($P=0.019$). HGB did not affected by any concentrations of HFCS ($P=0.970$). HFCS treatment significantly ($P=0.008$) elevated the PCV and MCV values of broilers in high dose groups rather than low dose group compared to the control group. The MCH from the erythrocyte indexes differentiated by HFCS application, which was increased by low dose while decreased by high dose, but they were individually similar with control group in terms of statistical evaluation ($P=0.149$). On the other hand, 10%-HFCS treatment remarkably reduced MCH and MCHC values compared to 5%-HFCS treatment ($P=0.001$) and non-treated control groups ($P=0.040$) in broilers, respectively.
Although HFCS application did not change total leukocyte counts (TLC) of broiler ($P=0.896$), it affected leukocyte subtypes of broiler chickens in various forms depending on the dose (Table 2). Monocyte was not affected from the 5% or 10% HFCS treatment ($P=0.593$). However, percentage of basophil significantly increased in the animals treated 5%-HFCS ($P=0.016$) while it was similar to control in the animals treated 10%-HFCS ($P=0.951$). High level dietary HFCS intake decreased the eosinophil values of broilers compared to both control and the animals applied 5% dietary HFCS ($P=0.035$). Effects of 5% or 10% HFCS

Table 1. The alterations of selected hematological parameters of broilers by 5% or 10% dietary intake of high fructose corn syrup.

	Control (n=12)			5% HFCS (n=12)			10% HFCS (n=12)			*P* value
	Mean	±	SE	Mean	±	SE	Mean	±	SE	
RBC (10^6/µL)	1.84	±	0.13[ab]	1.57	±	0.11[a]	2.28	±	0.24[b]	*P*=0.019
HGB (g/dL)	10.03	±	0.46	10.18	±	0.31	10.03	±	0.66	*P*=0.970
PCV (%)	28.92	±	0.90[a]	30.33	±	0.69[ab]	33.33	±	1.15[b]	*P*=0.007
MCV (fL)	23.13	±	1.26[a]	23.64	±	0.97[a]	28.52	±	1.47[b]	*P*=0.008
MCH (pg)	57.05	±	3.82[ab]	67.53	±	4.15[b]	46.52	±	3.57[a]	*P*=0.002
MCHC (g/dL)	34.76	±	1.25[b]	33.68	±	1.22[ab]	29.98	±	1.48[a]	*P*=0.039

[a,b]: Different letters in the same row represents statistical significancy as $P<0.05$ or $P<0.01$.

Table 2. The alterations in leukocyte subtypes of broiler fed with different concentrations of high fructose corn syrup.

	Control (n=12)			5% HFCS (n=12)			10% HFCS (n=12)			*P* value
	Mean	±	SE	Mean	±	SE	Mean	±	SE	
TLC (10^3/µL)	2.89	±	0.27	2.81	±	0.13	2.72	±	0.34	*P*=0.896
Basophil (%)	0.67	±	0.26[a]	3.08	±	0.89[b]	0.92	±	0.40[a]	*P*=0.011
Eosinophil (%)	1.83	±	0.60[b]	1.75	±	0.55[b]	0.08	±	0.08[a]	*P*=0.021
Heterophil (%)	21.75	±	1.74[b]	35.58	±	2.23[c]	11.75	±	1.78[a]	*P*=0.000
Lymphocyte (%)	75.50	±	1.69[b]	58.75	±	3.32[a]	86.50	±	1.89[c]	*P*=0.000
Monocyte (%)	0.25	±	0.18	0.83	±	0.66	0.75	±	0.30	*P*=0.593
H/L	0.29	±	0.03[a]	0.65	±	0.07[b]	0.15	±	0.03[a]	*P*=0.000

TLC: Total leukocyte counts, H/L: Heterophil / Lymphocyte. [a,b,c]: Different letters in the same row represent statistical significancy as $P<0.05$ or $P<0.001$.

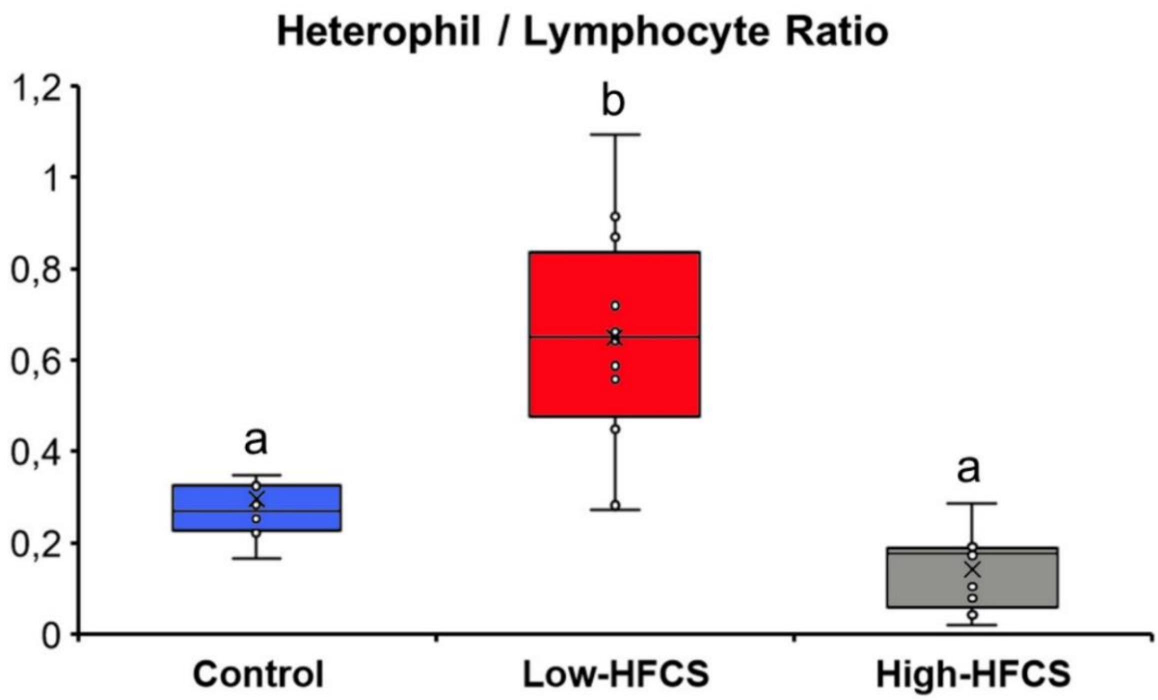

Figure 1. Effects of different concentrations (5% or 10%) of high fructose corn syrup treatments on heterophil/lymphocyte ratio in broiler chickens. [a,b]: Different letters on the boxplots represent statistical significancy as $P<0.001$.

treatment on heterophil and lymphocytes were the opposite of each other; the 5%-HFCS significantly increased heterophil and decreased lymphocyte while the 10%-HFCS decreased heterophil and increased lymphocyte values, respectively (*P*=0.000). Heterophil/lymphocyte (H/L) ratio did not change by high dietary HFCS intake but significantly increased by 5% dietary HFCS intake in broiler as seen in Figure 1 (*P*=0.000).

Discussion

Following the first development of HFCS at 1960s, usage of it in various foods and beverages has gradually increased over the years worldwide. Even the impact of HFCS on the health is debated intensely, excessive consumption of HFCS is reported to cause rising the incidence of disorders such as obesity, diabetes, cardiovascular diseases, and metabolic syndromes (Parker et al., 2010). Recently, fruit-derived by-products which contain fructose have been progressively utilized in poultry farming, however, the impact of fructose on the broiler chickens' metabolism is still unclear. The findings of our study conducted for this purpose firstly showed that different concentrations of HFCS intake by diet affected certain hematological parameters including heterophil and lymphocyte ratio in broiler chickens.

In the present study, rather than 5%-HFCS, 10%-HFCS treatment significantly elevated PCV and MCV, and increased RBC as well even it was insignificant compared to control group. Previous studies proved that MCV reduced in rats after oral administration of high fat/high fructose diet (Nurliyani et al., 2018), while it increased by fructose application in obese rats (Bakalov et al., 2021). In both reports it was found as a common result that RBC was not affected by fructose application. Fructose intake by HFCS may lead to hyperinsulinemia (Galderisi et al., 2019), which is associated with proliferation of erythrocytes and increasing of the RBC indices (Stonestreet et al., 1989). Stimulated red blood cell proliferation is most probably responsible for the increased MCV which can be resulted from reticulocytosis (Bakalov et al., 2021). This is also resulted in increased PCV as seen in our findings.

On the other hand, neither 5% nor 10%-HFCS affected HGB values of broiler chickens in the present study. In addition, the observed differences in MCH values by 5% or 10% HFCS treatment was not statistically significant. Similar results were also reported in rats fed with high fat-high fructose (HFHF) diet (Bakalov et al., 2021). However, MCHC values of broilers treated 10%-HFCS in our study significantly decreased compared to control groups' values. Although there are few different reports which were revealed any changes (Bakalov et al., 2021) or increments (Stonestreet et al., 1989) in MCHC values of rats followed by high fructose intake, the increased MCV and PCV values are most probably responsible for the reduced MCHC without non alterations in HGB of broiler chickens in the present study.

According to our findings, any concentrations of HFCS used in this study did not significantly change total leukocyte counts and monocyte percentage. However, other leukocyte subtypes and H/L ratio remarkably differed from the those of control group by 5% or 10% HFCS treatment. Findings of previous studies conducted in rats (Mihafu et al., 2020) and goats (Nurliyani et al., 2018), in which TLC and some of leukocyte subtypes did not change by high fat high fructose diet, are consistent with our results. Conversely, (Abd Elmonem and Ali 2011), reported that TLC and granulocytes decreased by consumption of fructose rich soft drink in balb/c mice. Nurliyani et al., (2018) also showed that HFHF decreased lymphocyte while increased neutrophil in goats as seen in our study following 5%-HFCS treatment. These alterations observed in percentage of heterophils, and lymphocytes may be resulted from HFCS which was shown as stimulator of stress and inflammation (Rippe and Angelopoulos 2013).

Furthermore, we found that unlike 10%-HFCS, 5%-HFCS elevated H/L ratio in broiler chickens. The H/L ratio is a common parameter that has been used for years to assess the stress status of chickens. It represents physiological changes that influence the long-term environmental conditions around the body. Because the number of lymphocytes and heterophils of broiler chickens decreased and increased, respectively, in response to stressor, the H/L ratio elevates as a stress indicator (Gross 1983). In this study, the source of stress leading to an increase in the H/L ratio may be acute allergic inflammation induced by 5%-HFCS, as the basophil percentage was also significantly increased in this group. Consistent with our results, Jung et al. (2018) et all reported that fructose can cause anaphylactic reaction by stimulating basophil activation in human drinking fructose-rich beverages. Another study found an association between frequent consumption of free-fructose drinks and allergy in children and adolescents (Yu et al., 2018).

On the other hand, 10%-HFCS treatment did not affect basophils and H/L ratio but significantly increased lymphocytes. The reason of this increment observed in the percentage of lymphocyte in our study may be intestinal damage caused by HFCS. Moughaizel et al., (2022) showed that high fructose consumption via HFCS sweetened drinks led to disruption of intestinal barrier and increased gut permeability. In case of intestinal barrier damage, undigested foods leak into the bloodstream. As a result of this, lymphocytes located in the small intestine wall as an agent of immune system elevates to induce systemic inflammatory processes (Stewart et al., 2017; Arnone et al., 2022).

Conclusion

In conclusion, this study demonstrated for the first time that HFCS treatment can affect hematological parameters of broiler chickens. According to our results, low (5%) HFCS treatment increased basophil and H/L ratio (consistent with the changes observed in heterophil and lymphocytes) while high (10%) HFCS treatment increased PCV, MCV, and lymphocytes. Thus, we suggest that low-dose HFCS consumption may lead to allergic reactions, while high-dose HFCS consumption may cause disruption of the intestinal wall, but this needs to be comprehensively elucidated by microscopic investigation in further studies.

Ethical Statement

The scientific design of this study was approved by the Local Ethical Committee of Kırıkkale University with the decision date and number of 2020-06/06.

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Author Contributions

First Author (TA): Writing -review and editing, Data Curation, Resources; Second Author (GŞ): Conceptualization, Writing -review and editing, Data Curation, Investigation, Supervision, Project Administration, Methodology, Visualization and Writing -original draft; Third Author (RK): Conceptualization, Supervision, Funding Acquisition, Resources, Writing -review and editing, Formal Analysis.

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