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BLOOD HEMATOLOGICAL, IMMUNITY AND ORGANS LYMPHOID OF FEMALE SENTUL CHICKEN FED DIETS SUPPLEMENTED WITH SAFFLOWER OIL (*CARTHAMUS TINCTORIUS*. L) AND INOSITOL

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ABSTRACT

This study evaluated the blood hematological, immunity, organs lymphoid of combinations safflower oil (SO) and inositol (IN) in female Sentul chicken. A total of 81 birds, was 16 weeks old which were raised throughout 8 weeks. Nine experimental diets were formulated, control diet = SO 0% + IN 0%, SO = 0,5%, IN = 0,5%, SO = 1%, IN = 1%, SO 0,5 % + IN 0,5%, SO 0,5% +IN 1%, SO 1% + IN 0,5%, SO 1% +IN 1%. Used research a completely random design; nine experimental with three repetitions for each replicate of three birds. Were the variables being feed consumption, hematological, titer antibodies against New castle disease, avian influenza, and lymphoidorgans. The results showed the treatments had no significant ($p<0.05$) feed consumption on the first week of birds while in the second to the six-week application of different levels supplementation safflower oil and inositol application affected was significantly ($p>0.05$) affected feed consumption and did not significant difference ($p >0.05$) of hematological, titer antibody of ND,In contrast, AI was highly significantly different ($p < 0.01$), the spleen and thymus did not have a significant difference (P>0, 05) while the relative weight of bursa of Fabricius was high significantly (P<0, 05). The application supplementation safflower oil with inositol had no negative effect on hematological, still produced relatively similar of blood, increased the feed consumption at (IN 0.5 %), weight in bursa Fabricius at (SO 1%+IN 1%) , increased response immune indices of titer antibody at (SO1%)(IN 1%)(SO 0.5%+IN 1%) this indicates healthy of chicken.

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INTRODUCTION

In many areas of Indonesia, local chickens are also raised, which play an important role in meal production, often providing the primary source of dietary protein in people's eating regimens, they are commonly known as "non-breed chickens (ayamkampung) to differentiate nearby chickens from commercial poultry breeds such as extensively recognized breeds of Cobb, Hubbard, Hybro, Isa, Hyline, and Hisex Indonesian native chicken interestingly have species physical attribute are grouped into at least 34 breeds or awesome organizations of nearby chicken particularly. Any of the chickens are used for non-nutrition reasons, such as religious ritual contributions, excellence – voice and plumes, and rooster – fighting and sure

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Kampung chickens are the most well-known and kept nearly all through the whole nation (Henuk and Bailey, 2014). Like an egg and meat kind, it is a special sort of local chicken. Hidayat and Sopiyana (2010) recognized that Sentul chicken, an Indonesian animal-genetic resource, had a good potential to be a commercial commodity. The importance of local breeds of fowl bird for the rural economic system in growing and undersized international locations broadly speaking in Asia and Africa is high. Problems occur in reducing the production of eggs and the development of the local strain and the face of diseases, and in declining immunity and health status and mortality, as well as in increasing and generating cycles of treatment and breeding, and in need of chicken for certain services and intensive care systems and supplements to boost production and productivity and increase immunity (FAO, 2010). The hematological and immune system plays a key role in supporting the health and productivity of chickens. Organs related to the immune system of broiler chickens are lymphoid organs because they function in producing antibodies

(Baktiningsih *et al*., 2013), Few findings suggest that body weight gain has a positive effect with inositol, there is little understanding of the causes for this growth improvement, current awareness indicates that inositol has several important biochemical roles within the body, with many tissues capable of synthesizing and catabolizing this poly. (Lee and Bedford. 2016). While Safflower seed oil is abundant in 75-78 percent linoleic acid, which plays an important role in lowering blood cholesterol levels and enhancing efficiency and health status, immune poultry diets have increased starch nutritional value, fat digestibility, and digestibility. (Sanz *et al*., 2000). The paper aimed to evaluate blood hematological, immunity, and organ lymphoid of local chickens (female Sentul chickens) fed diet supplements with safflower oil and inositol.

MATERIALS AND METHODS

According to the approximate analysis of safflower oil, nine experimental dietaries were formulated to meet the nutrients requirements of local chicken (NRC, 1994). The control diet without safflower oil (SO) and inositol (IN) Safflower oil and inositol were formulated and eight experimental diets were formulated to contain. Based on safflower oil and inositol, at different levels, samples of experimental diets were analyzed for proximate chemical composition by methods of analysis stated by AOAC (2006). All experimental dietary were in isonutrient and isocaloric conditions. The diets were arranged to the feed protein content as 17% and energy metabolic at 2800 cal/g, Corn, rice brain, Soybean meal, fish meal, Sawit oil, CaCo3, Topmix Lysine Methionine (Table 1). According to AOAC (2006), the composition of the feed was analyzed, feed and water were given ad libitum. The Supplementation feed treatments consisted: control = SO 0% + IN 0%, SO = 0,5%, IN = 0,5%, SO = 1%, IN = 1%, SO 0,5 % + IN 0,5%, SO 0,5% +IN 1%, SO 1% + IN 0,5%, SO 1% +IN 1%.

Experiment management: The experiment was conducted at the Department of Poultry Faculty of Animal Science University of JenderalSoedirman, Purwokerto, Indonesia. This study used 81 bird female, which was 16 weeks old which were raised throughout (8 weeks) including one week for adaptation which was divided randomly into nine experimental with three repetitions each replicates three bird. Randomly sited battery cages sized 26 × 36 × 36 cm 3 were used, and feed and water were placed in front of the cages.

Feed consumption: Feed consumption is calculated based on the amount of feed consumed per day by looking at the recording every week

Measurement of Hematological: After eight weeks, blood samples were obtained from the vessel underwings, three milliliters tubes containing blood anticoagulants is packed with samples taken. (EDTA/Ethylene Di-amine Tetra Acetic acid) 3 ml of the blood sample was taken from vena axillaries, put in an EDTA-filled tube, kept in an ice-filled flask, the carried to the laboratory for further examination. The amount of erythrocyte of RBC (mil/ml) was counted using a hemocytometer with Hagem dilution (Zhang *et al*., 2007). The amount of leukocyte (WBC) and differential leukocyte was measured using the blood swap method colored with Wright reagent using Hematek Stain Pak. 100 WBC per blood sample was examined using Nikon microscope with 400x magnification, and so were the identification of heterophile, lymphocyte, monocyte, eosinophile, and basophile. The total heterophile and eosinophile amount was counted using NeubauerHemacytometer and Nikon microscope with 100× magnification. Cell coloring used phloxine B propylene air glycol. The total amount of lymphocyte, monocyte, and basophile was indirectly determined from the percentage calculation of cell and total heterophile and eosinophile (Chowdhury *et al*., 2005), the calculation of the heterophile / lymphocyte ratio is as follows:

Measurement of immunity: Viral antibodies against New castle disease (ND) and Avian influenza (AI) viruses were determined using the haemagglutination inhibition (HI) was done according to Takatsy (1956) test on samples from two birds in each replicate at day 42.

Measurement of organs lymphoid: The thymus, spleen, and Bursa were separated and individually weighed. The relative weight of lymphoid organs was expressed as relative to live body weight and percentage Organs Lymphoid By (weight organs lymphoid /bodyweight X 100%).

Data analysis: Data were analyzed using ANOVA Complete Random Design (CRD) if significant differences in treatments exist. It was supported by the Honestly Significant Differences (Steel and Torrie, 1994).

RESULTS AND DISCUSSION

Supplementation Safflower Oil and Inositol on feed consumptionof female Sentul Chicken: Results regarding feed consumption of female Sentul chicken presented in (Table 3) the statistical analysis recorded that combination of safflower oil and inositol application affected non significantly ($p<0.05$) on the first week of feed consumption, while in the second to six weeks had significantly ($p>0.05$) of female Sentul chicken, which high level recorded on the fifth week at (IN 0.5 %) 241.68±/g compared to other treatment and a lower feed consumption on the fourth week in treatment (SO 0.5%)142.78/g These results agree agrees with the findings of Hosseini *et al*. (2008) found that the effect of inclusion of different levels, (0, 4, 7 and 10%) of safflower seed on the layer of feed intake and feed conversion ratio. Rodriguez *et al*., (2005), who reported no significant differences in weight gain, feed intake and feed utilization among the chicks that received control diet and those fed diets with increasing level of full-fat safflower seeds from 5 to 25% of diet. These findings were also consistent with the results of Selvaraj *et al*, (2004) who used various levels of full-fat safflower seeds (0%, 5%, 10%, 15% and 20%) and reported that weight gain and feed consumption were not affected by the full-fat safflower seeds inclusion. Cowieson *et al*. (2013) the ability for the addition of inositol to broiler diets to boost growth efficiency was shown. Supplementation gave greater feed intake at (IN 0.5 %)(IN 1%)(SO, 1%+IN 0.5%) feed consumption is positively correlated to egg production, BW gain because feed consumption in chickens greatly impacts production since its key in determining the nutrient intake levels, the high recorded in treatment possibly due to the combination of safflower oil given to maintain normal body weight and safflower oil is rich in linoleic acid 75 -78%, which can provide health benefits by maintaining the body cells which helped to increase the feed consumption . Mueller *et al*. (2000) showed that supplementation of (CLA) in Iso-energy diets with a strongly positive energy balance has no marked effect on total lipid metabolism, but lightly enhanced deposition of protein is evident. Pirgozliev *et al*., (2007) reported an improvement in BWG and feed intake with inositol supplementation at 0.25% to a low P diet in broilers from d 7-17, Inositol supplementation was optimal in the absorption of protein in layer

Supplementation Safflower Oil and Inositol on Hematological Parameters of FemaleSentul Chicken: The results of hematological parameters of female Sentul chicken are presented in (Table 4). Hematological parameters observed in this study include the erythrocytes (mil/µl), leukocytes (cell/µl), H/b (g/dL), heterophile (%), lymphocytes (%) and monocytes (%) PCV (%), TTP, H/L. There was no significant effect ($P>0.05$) in the variation of hematological parameters in this study. The of supplementation safflower oil and inositol to the chicken's hematological status made the physiological processes run well, leading to good health and optimum production.

The amount of erythrocytes in the chicken, there were normal for females. Kusumawati (2000) states that the number of erythrocytes in poultry is 1.25 - 4.50x 106 / µl. Dharmawan (2002) added that the normal range of erythrocyte counts in chickens was 2.3-3.5 x 106 / µl. Talebi *et al*. (2005) reported that the total erythrocyte of native chicken was 2.17–2.86 (106/mm3) while other findings varied from 2.49±0.38–2.83±0.64 (106/mm3)

Table 1. Feed compositions Female Sentul chicken

Ingredient	Control	SO 0.5%	IN 0.5 %	SO, 1%	IN 1%	SO 0.5%+IN 0.5%	SO 0.5%+IN 1%	SO, 1%+IN 0.5%	SO, 1%+IN 1%
	%								
Corn	55	55	55	55	55	55	55	55	55
Rice bran	22	22	22	22	22	22	22	22	22
Soybean meal	15	15	15	15	15	15	15	15	15
Fish meal	5	5	5	5	5	5	5	5	5
Sawit oil	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
CaCo3	1	1	1	1	1	1	1	1	1
Topmix	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Lysin	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Methionin	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Safflower oil	0	0,5	1	0	0	0,5	0,5	1	1
Inositol	0	0	0	0,5	1	0,5	1	0,5	1
Total	100	100,5	101	100,5	101	101	101,5	101,5	102

Note: Results of analysis of the Laboratory of Animal Nutrition and Forage (2020) Faculty of Animal Husbandry, University of Jenderral Soedirman. The nutrient content of the ingredients refers to the NRC table (1994) according to the feed requirement of broilers. SO: Safflower Oil IN: Inositol

Table 2. Nutrien composition of Female Sentul chicken

Nutrien composition	Control	SO 0.5%	IN 0.5 %	SO1%	IN 1%	SO 0.5%+IN 0.5%	SO 0.5%+IN 1%	SO 1%+IN 0.5%	SO 1%+IN 1%
	%								
Crude Protein (%)	17,32	17,32	17,32	17,32	17,32	17,32	17,32	17,32	17,32
Crudefat (%)	4,00	4,00	4,00	4,00	4,00	4,00	4,00	4,00	4,00
Crudefiber(%)	4,75	4,745	4,75	4,75	4,75	4,75	4,75	4,75	4,75
ME(kkal/kg)	2.884,0	2.890,8	2.897,6	2.884,0	2.884,0	2.890,8	2.890,8	2.897,6	2.897,6
Calcium (%)	1,28	1,2756	1,28	1,28	1,28	1,28	1,28	1,28	1,28
Phosphor (%)	0,38	0,3779	0,38	0,38	0,38	0,38	0,38	0,38	0,38
Lysin (%)	1,11	1,1074	1,11	1,11	1,11	1,11	1,11	1,11	1,11
Methionin (%)	0,67	0,6708	0,67	0,67	0,67	0,67	0,67	0,67	0,67

ME: Metablasim Energy

Hidayat *et al.,* (2016),safflower oil containing oleic and linolenic fatty acids can function as a producer of arachidonic acid which is a precursor to prostaglandin hormones that can play a role in immune regulation when the chicken is in a healthy condition, its body will stabilize the value of erythrocytes according to Fassah and Lilis (2016). Inositol is a B-complex vitamin that plays a role in erythropoiesis or the final maturation stage of erythrocytes. The appropriate erythrocyte maturation stage results in a normal number of erythrocytes folic acid: vitamin B9 and vitamin B12 needed for the formation of red blood cells (Guyton and Hall 2008). Irianti *et al.* (2014) stated that erythrocytes are influenced by several factors including gender, age, body condition, and blood cell size, and stress conditions.

The results of the average number of leukocytes obtained shows that the supply of safflower oil and inositol does not give a negative response and tends to be able to maintain the leukocyte count within normal limits, this is supported by Abdullah *et al.* (2018) who stated that the number of leukocytes in the blood of normal native chickens aged 3-12 weeks was in the range of 7,000-21,000 / mm³. Magna *et al* (2012) stated that the addition of inositol with a level of 1-2.5% in the ration can increase the protein content in the blood so that it is directly proportional to the increase in the number of leukocytes. The results of this study female Sentul chickens were in a healthy condition as the percentage of heterophile.Hendro *et al.* (2013) stated that the normal percentage of heterophile in broiler chickens is 20-40%. factors that influence the level of heterophile include environmental conditions and genetics. Baratawidjaja *et al.* (2009) stated that heterophile work by attacking pathogens through independent oxygen pathways. The percentage of lymphocytes within the normal, although there is a high percentage of lymphocytes in Sentul chicken, however, is within the normal range, which poultry generally 42– 66% according to (Harahap. 2014).High lymphocyte count in femaleSentul chicken blood treated with (SO 0.5%+IN 0.5%) 67 % and (SO 0.5%) 68% is thought to be due to B vitamins and essential fatty acids in eicosanoid compounds found in inositol and safflower oil which help stimulate the proliferation process to increase the number of lymphocytes because B vitamins and essential fatty acids work synergistically in activating lymphocytes (Agustanti, 2014). Safflower oil supplementation with 0.5% and 1.0% inositol tends to increase monocytes due to the essential fatty acid content in safflower oil which helps in the formation of eicosanoid compounds that play a role in maintaining endurance,the standard of monocytes in broiler chicken blood is 3 - 10% (Eroschenko and Di Fiore 2013). Lokopirnasari and Yulianto (2014) added that the number of monocytes in chicken blood has a normal range of 0-30%. Davison (2014) reported the main role of monocytes in the immune system is that of responding to inflammatory stimuli, mobilizing macrophages against antigens, and releasing substances that can reduce inflammation. The hemoglobin level of femaleSentul chickens in each treatment is still within the normal range according to Samour (2006) the normal hemoglobin levels in chickens range from 10.2-15.1 g / dL. Safflower oil and Inositol plays a role in stabilizing hemoglobin levels where oleic acid and linoleic acid in the form of unsaturated fatty acids function in accelerating the metabolic process to absorb protein which is the main component of hemoglobin,the high and low levels of hemoglobin are influenced by several factors such as protein content in rations, age, environment, and cage conditions (Alfian, *et al.*, 2017). Female Sentul chickens are still within the normal range of PCV is 22-35 % according to Bounous and Stedman (2000), also PCV was not influenced by the application of supplementation safflower oil and inositol. Abdi-Hachesoo *et al*., (2011) the difference in hematological values among local chickens reared in the different regions shows the potential health status, that there are different physiological properties (age and activity), environmental factors (temperature and humidity), and feed composition. The results showed no significant difference in total protein plasma this result disagrees with the study of Ibrahim Albokhadaim (2012), these variations are more dynamic in chicken and are usually related to intensive metabolic processes and changes in nutrition.The plasma protein functions as body protein reserve, circulates non-statically,

Table 3. Supplementation of Safflower Oil and Inositol on feed consumptionof female Sentul Chicken

Feed consumption (age /weeks)	Treatment								
	Control	SO 0.5%	IN 0.5 %	SO, 1%	IN 1%	SO 0.5%+IN 0.5%	SO 0.5%+IN 1%	SO, 1%+IN 0.5%	SO, 1%+IN 1%
	Gram								
1	230.76±13.96	215.45±27.29	210.34±26.60	225.86±17.63	225.17±26.79	229.12±16.63	221.82±18.94	220.98±10.17	200.61±25.96
2	235.76±8.95 ^{a}	208.68±28.89 ^{a}	218.55±22.19 ^{a}	212.45±28.60^{a}	208.95±23.66 ^{a}	234.09±6.80 ^{a}	208.95±17.18 ^{a}	228.80±13.14 ^{a}	209.27±14.31 ^{a}
3	214.86±10.67^{ab}	182.09±17.19 ^{a}	220.51±22.92^{c}	180.47±17.40^{a}	202.98±22.34 ^{ab}	215.90±4.63^{c}	186.47±16.40 ^{ab}	211.87±10.65^{bc}	206.28±18.04 ^{ab}
4	222.89±12.81^{a}	142.78±24.58^{c}	220.94±13.70^{c}	189.52±16.21^{b}	205.14±17.94^{bc}	228.87±6.49^{c}	216.01±14.32^{bc}	233.31±28.89^{c}	216.04±15.00^{bc}
5	234.84±7.19^{c}	152.28±8.64^{a}	241.68±36.66^{c}	194.13±16.43^{b}	220.81±8.14^{bc}	228.11±7.28^{c}	214.96±6.17^{bc}	210.36±6.82^{bc}	199.32±17.69^{b}
6	225.03±8.41^{c}	150.38±8.98^{a}	223.53±19.73^{bc}	199.42±10.37^{b}	226.53±9.94^{c}	231.41±6.22^{c}	213.70±16.40^{bc}	206.01±15.80^{bc}	210.61±24.33^{bc}

Table 4. The Averages of Supplementation Safflower oil And Inositol on Blood Hematological of Female Sentul Chicken

Parameters	Treatment								
	Control	SO 0.5%	IN 0.5 %	SO1%	IN 1%	SO 0.5%+IN 0.5%	SO 0.5%+IN 1%	SO 1%+IN 0.5%	SO 1%+IN 1%
Erythrocytes(mil/µl)	2.2±0.33	2.5±0.17	2.3±0.49	2.6±0.13	2.8±0.46	2.3±0.42	2.4±0.39	2.1±0.38	2.5±0.15
Leukocytes (cell/µl)	8016.67±1833.26	9350.00±3785.83	10316.67±366822	9783.33±2353.90	8700.00±2971.53	10050.00±3518.17	10050.00±1710.99	8183.33±2322.89	9200.00±1408.01
H/b (g/dL)	7.30±0.17	7.57±0.40	7.87±0.61	7.57±0.40	8.60±0.35	7.67±0.29	8.10±0.52	8.10±0.52	8.10±0.52
Heterophile (%)	29.00±7.5	27.67±6.5	34.00±6.6	43.33±7.5	34.00±9.5	38.67±7.0	27.00±10.4	39.33±5.7	37.00±14.8
Lymphocytes (%)	64.67±7.23	68.33±6.81	58.67±8.50	51.00±9.17	57.00±10.58	53.67±6.81	65.33±8.33	56.33±5.77	57.00±16.64
Monocytes (%)	6.00±2.00	4.00±1.73	7.33±2.52	5.00±100	8.00±300	7.00±458	7.67±252	4.33±1.53	5.33±2.31
PCV (%)	22.00±2.00	23.67±1.53	24.00±3.61	25.33±1.53	27.00±3.46	22.33±2.52	24.67±4.04	23.67±3.51	24.67±0.58
TPP (g/dL)	3.13±0.12	3.47±0.50	3.33±0.12	3.80±0.53	3.93±0.64	4.00±0.69	3.53±0.50	4.67±0.31	3.73±0.64
H/L (%)	.46	.41	.60	.89	.70	.74	.43	.71	.73

H/b: hemoglobin; PCV: packed cell volume ; TPP: total protein plasma ;H/L: Ratio Heterophile Lymphocytes ; NS: No significant; Mean ±: standard deviation

Table 5. The Mean Value Titer of Antibody Titer For ND, AI of Supplementation Safflower oil and Inositol of Female Sentul Chicken

Parameters	Treatment								
	Control	SO 0.5%	IN 0.5 %	SO1%	IN 1%	SO 0.5%+IN 0.5%	SO 0.5%+IN 1%	SO 1%+IN 0.5%	SO 1%+IN 1%
Titer ND	25.33±0.58	26.67±2.08	25.33±1.53	22.67±3.06	26.00±1.73	26.67±0.58	23.67±2.52	26.00±0.00	25.33±1.53
Titer AI	27.67±1.53^{a}	27.67±0.58^{a}	32.67±8.14^{a}	196.67±15.28^{b}	200.0±17.32^{b}	28.67±0.58^{a}	170.00±45.83^{b}	26.33±1.15^{a}	28.00±1.00^{a}

ND: Newcastle Disease
AI: Avian influenza
Mean ±: standard deviation
NS: No significant
a,b, Means in the same row with different superscript differ significantly ($p<0.05$)

Table 6. The Averages of Supplementation Safflower oil And Inositol on Organ Lymphoid (Thymus, Bursa Fabricius, Spleen) of Female Sentul Chicken

Parameters						
	Thymus Bursa Fabriciusspleen					
Treatment	(g)	(%)	(g)	(%)	(g)	(%)
Ccontrol	.41±.01	.033	15.43±.31 [ab]	1.23	6.60±.87	.52
SO 0.5%	.20±.10	.015	14.93±2.54 [ab]	1.10	6.60±.52	.49
IN 0.5 %	.22±.11	.015	17.43±.90 [ab]	1.15	5.03±2.86	.33
SO1%	.32±.08	.023	17.43±.67 [ab]	1.28	6.00±2.09	.44
IN 1%	.20±.09	.016	18.77±.[75 bc]	1.54	2.13±.97	.18
SO 0.5%+IN 0.5%	.28±.04	.022	16.27±1.86 [ab]	1.25	5.33±1.12	.42
SO 0.5%+IN 1%	.41±.05	.028	15.80±2.25 [a]	1.05	5.60±.87	.38
SO 1%+IN 0.5%	.32±.06	.021	15.83±1.70 [a]	1.01	4.73±.49	.30
SO 1%+IN 1%	.35±.10	.025	23.63±2.87[c]	1.74	3.20±.78	.24

Mean ±: standard deviation NS: No significant ** Mean values with different superscripts in the same row were significantly at ($p<0.01$) *Mean values with different superscripts in the same row were significantly at ($p<0.05$) a,b, c Means in the same row with different superscript differ significantly ($p<0.05$)

exchanges continuously with unstable tissue reserve whose amount is equal with circulating protein to make a dynamic balance. Tóthová*et al.* (2019) stated that the fattening and age of broilers influence not only the production patterns, metabolic processes, and lipid and mineral profile but also the parameters of protein profile. The value of the H/L ratio for each treatment is considered normal with an average of0.63 of females. According to Emadi *et al.*, (2007), the level of resistance in poultry can be determined by the value of the H / L ratio: around 0.2 (low), 0.5 (normal), and 0.8 (high) against environmental adaptation. The increase in the value of the H / L ratio in chickens is influenced by environmental temperature. Aengwanich and Chinrasri (2002) reported that chickens under stress will experience a decrease in the number of lymphocytes and an increase in the number of heterophile so that the ratio between heterophils and lymphocytes increases.

The Mean Value of Antibody Titer For ND, AI of Supplementation Safflower Oil and Inositol to Female Sentul Chicken: The result in (Table 5) shows AI and ND at 42 day-olds of antibody titers. The results were not significantly different ($P<0.05$) at titer ND, while AI was significantly different ($p < 0.05$) which was higher at (SO, 1%)196.66 and (IN 1%) 200.0 (SO 0.5%+IN 1%) 170.0, these results agree with Wang (2004) reported that chickens fed on a diet supplemented with fish oil at a level of 50 g/kg showed a higher production of antibodies (IgM and IgG) and globulins in the serum. the titers increased mostly due to the response to the vaccination and the positive effect on immune indices, the antibody responses could also be attributed to the high contents of fatty acids of which polyunsaturated fatty acids, particularly linoleic acid are dominant (Attia *et al*., 2017). Sentul chickens have been classified as disease resistant and with good immunity, increasing the level of unsaturated fatty acids improves the immune response by stimulating the macrophage cells which contains a combination of safflower oil and inositol. The diet contains n-3 fatty acids such as linoleic as well as eicosatetraenoic that act as modulators of the rate of conversion of arachidonic fatty acid to eicosanoids. The immune response and oxidative mechanisms are interlinked and affect one another, hence modulation of one can impact the other (Elwan *et al*., 2019). Improving genetic resistance to ND and AI in the breeding of local chicken different according to environmental.

Supplementation Safflower of Oil and Inositol on Organ Lymphoid of Female Sentul Chicken: The values of lymphoid organ weight are shown in (Table 6) The result showed weights of the spleen and thymus did not differ significantly ($P>0,05$), while the relative weight of the bursa of Fabricius was high significantly ($P<0,05$), this result agrees with that of Latif *et al*. (2014), lower than that found of Attia *et al*. (2017), the relative weights of males and females were similar to Baktiningsih *et al*., (2013). which the high recorded at (SO 1%+IN 1%) 23.63±2.87g of Bursa Fabricius may due to safflower oil with contents of polyunsaturated fatty acids stimulates the growth of immune tissue (thymus, spleen, and bursa), which plays an important role in T and B cell development and generation of the cellular and immunoglobulin repertoire. According to Boehm and Bleul (2007) inositol functions to increase fat and protein liver participation in metabolization and contributes to immunity by synthesizing accessory proteins.

The lymphoid organs play an important role in defense mechanisms against microorganisms, previous research has shown that the growth of lymphoid tissue may represent the immune state (Grasman, 2002; Smith and Hunt, 2004; Akter *et al*., 2006). Al–Khalifa *et al*. (2012) reported that feeding n-3 PUFA rich diets (30, 50, and 60 g/kg fish oil) did not affect the relative weight of the spleen. But significantly increased the thymus weights in broilers fed diet with 50 g/kg fish oil and significantly lower the bursa weights in broilers fed diets containing 50 and 60 g/kg fish oil.

Conclusion

The concentration of safflower oil with inositol had no negative effect on hematological, still produced relatively similar of blood indicates that healthy of chicken, increased the feed consumption at (IN 0.5 %), weight in bursa Fabricius at (SO 1%+IN 1%) , increased response immune indices of titer antibody at (SO1%)(IN 1%)(SO 0.5%+IN 1%) of Avian influenza, this indicates healthy of chicken.

Conflicts of Interest: All authors declare no conflicts of interest

ABBREVIATIONS

Abbreviation	Meaning
SO	Safflower oil
IN	Inositol
CaCo3	Calcium carbonate
RBC	Red blood cell
WBC	White blood cell
EDTA	Ethylene Di-amine Tetra Acetic acid
AI	Avian influenza
ND	New castle
HI	Haemagglutination inhibition
CRD	Complete Random Design
PCV	Packed cell volume
T/H	Total hemoglobin
H/L	the ratio of heterophils and lymphocytes
CLA	Conjugated linoleic acids
TTP	total protein plasma
IgM	Immunoglobulin M
IgG	Immunoglobulin G

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