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The role of dietary lignocellulose inclusion with or without probiotic supplementation on some immune and blood biochemical parametres in broiler chickens

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ABSTRACT

Two hundred and forty, one-day old Avian broiler chicks were used to investigate the immune role of dietary Lignocellulose (LC) inclusion with or without probiotic supplementation and their effect on some blood biochemical parameters in broiler chickens. Experimental treatments consisted of 3×2 factorial arrangements with 3 levels of LC without or with probiotic to formulate six different experimental groups. Groups 1, 3, and 5 were fed on the basal diet with dietary LC inclusion at 0, 0.5%, and 1.0% respectively, while groups 2, 4 and 6 were fed on the previously mentioned design with probiotic at 100gm/ton. The obtained results revealed that dietary inclusion of either LC or probiotic increased (P≥0.05) WBCs count, LC combination with probiotic majored the WBCS compared with LC free groups. LC inclusion without probiotic reduced (P≥0.05) RBCs count, Hb and PCV. Meanwhile probiotic supplementation in combination with LC non-significantly improved these 3 parameters compared with probiotic free groups. Dietary inclusion of LC and / or probiotic minify lymphocyte ($p<0.05$) but increased (P≥0.05) heterophil %, phagocytic and phagocytic index and level of the antibody titer against Newcastle disease. LC and / or probiotic addition led to better serum lipid profile (decreased LDL, VLDL and CHO/HDL and increased HDL) and resulted in elevation of serum glucose. dietary inclusion of either LC or probiotic had no significant effect on kidney and liver function related enzymes. The probiotic supplemented diet with 0.5% LC inclusion recorded the lowest serum creatinine level. We concluded that either LC or probiotic enhanced immune response parameters and lipid profile (increased HDL and lowered cholestrol) and their combination magnified this enhancement. LC (0.5% - 1.0%) with probiotic supplemented diet revealed the best blood picture and lipid profile, the highest heterophil %.

1. INTRODUCTION

In most countries, the major animal protein produced and consumed is poultry meat which requires intensive broilers production to meet all the needs. intensive rearing of poultry causes stress on the chicks, so lowering the immune response and increase the pathogens colonization in the intestine (O'Dea et al., 2006). As a result, the antimicrobial agents been used for treatment and protection poultry from microbial diseases. Moreover, the extensive use of antibiotics use may generate antibiotic-resistant bacteria and residue in animal products and environment that also negatively affect the consumer leading to great risk to public health (Pelicano et al., 2004; Shivaramaiah et al., 2011; Sethiya, 2016), thus has brought about a world-wide call for banning or limiting the antibiotic growth promoter (Wallingaand Burch 2013; Smith, 2014). Pathogenic bacteria controlling without using the harmful synthetic drug or antibiotics becomes challenging (Ohimain and Ofongo, 2012). Therefore a severe and urgent demand to control or prevent pathogens using safe and practical alternatives immunomodulator (Mateos et al., 2002). Probiotic and dietary fibers influence the various immune responses of poultry

so can be used as potential substitute for antibiotics(Lowry et al., 2005; Smith, 2014; Sadeghi et al., 2015; Sethiya, 2016). Probiotics are a culture of live microorganisms that have beneficial effects on health of animal and poultry (Mahfuz et al., 2017). Previous researchers investigated the probiotic cholesterol-lowering effects, plasma immunoglobulins increasing, pathogen inhibition and immunity improving in poultry (Panda et al., 2008; Mountzouris et al., 2009; Ooi and Liong, 2010; Tang et al., 2017).The recently studies of Dong et al. (2020), Ebeid et al. (2021), Xu et al. (2021) and Mohamed et al. (2022) revealed that that dietary supplementation of probiotics (Bacillus subtilis) to broiler feed elevated serum total protein and antioxidant enzymes (catalase, superoxide dismutase glutathione peroxidase) and decreased of MDA concentration also boosted cell-mediated immune response through increasing the levels of immunoglobulins (IgE, IgA, IgG and IgM), anti-inflammatory IL-10 and TGF-β and proinflammatory IL-1b in broiler serum. Dietary fiber can be used as a cost-effective nutrient to modulate the poultry immune system. The consumption of an appropriate dietary fiber amount improves gut development and health (Jha and Mishra, 2021). Among various dietary fiber used, eubiotic lignocellulose (LC) that made from special pure wood recently used as high-quality fiber source for chickens. It is composed of structural carbohydrates (cellulose, hemicelluloses) and aromatic polymers (lignin). Previous studies revealed that small amounts of dietary LC inclusion for chickens promotes the growth of beneficial bacteria which produce short-chain fatty acids and lactic acid that decrease the pathogenic bacteria so maintain eubiosis in the intestine thus beneficially affect animal health (Saki et al., 2010; Bogusławska-Tryk et al., 2015; Youssef and Kamphues, 2018). Dietary LC addition exhibited positive effects serum immunoglobulin concentrations and cecal microflora (Sozcu, 2019; Sozcu and Ipek, 2020).

Generally the effect of lignocellulose and probiotic on immunity has been investigated separately. Nearly no studies have been conducted on the impacts of adding eubiotic lignocellulose interacting with probiotic in the diets on immune response of broilers chickens. We assumed that lignocellulose and probiotic may have a synergistic effect and help the action of each other. Our aims were to evaluate the impacts of using different levels of lignocellulose with or without probiotic supplement in the diets on immune response and some blood biochemical parameters in broilers chickens.

2. Materials and Methods

2.1. Experimental design, housing and feeding:

Two hundred and forty, one-day old Avian broiler chicks were used in this experiment. They were obtained from a local Egyptian private hatchery. The broiler chicks were randomly allotted into 6 equal groups (40 chicks/group), each group consisted of 2 replicates containing 20 birds per replicate. The chicks were housed in a clean well-ventilated room previously fumigated with formalin and potassium permanganate. The room was provided with electric heaters to adjust the environmental temperature according to the age of the birds. Feed and water were providedad libitum. Diets were formulated to provide the recommended requirements for broiler according to poultry NRC, 1994 (without added antibiotics, or growth promoters). Broiler chicks were fed on starter, grower, and finisher diets during the first two weeks, 3-4 weeks, and 5-6 weeks of the experiment respectively. Experimental treatments consisted of 3×2 factorial arrangements with 3 levels of lignocellulose (LC) without or with probiotic supplementation to formulate six different experimental diets. Groups 1, 3, and 5 were fed on the basal diet with LC inclusion at 0, 0.5%, and 1% respectively. Groups 2, 4 and 6 were fed on the previously mentioned design with probiotic supplementation. The applied experimental design is illustrated in table (1).

Table (1): The applied experimental design.

Groups	Lignoellulose inclusion level*	Probiotic supplementation**
1	0	--
2	0	++
3	0.5 %	--
4	0.5 %	++
5	1 %	--
6	1 %	++

*** (** produced by jelu-werk company,Germany**) ,****Probiotics supplemented as 100 g per ton feed **(**produced by United Biomed UBK group,Clostat HCSP , Bacillus subtilis6 $2x10^{11}$ cfu /g).

2.2. Immune response measurements:

2.2.1. Samples collection: At the 42nd days of age (and at 14th, 28th and 42nd days of age for haemagglutination inhibition test and glucose determination), blood samples were collected from six birds / group (three from each replicate), which was left to coagulate at the room temperature. Separation of the serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 minutes. The clear serum was kept in a freezer (-20 0C) until analysis. In clean dry vials containing anticoagulant (0.1ml sodium citrate 3.8%), another blood samples were received for determination of some blood pictures; total leukocytic counts (WBCs), red blood cells (RBCs) counts, hemoglobin (Hb), packed cell volume (PCV). Also for differential leukocytes count, phagocytic activity (PA) and phagocytic index (PI).

2.2.2. Total leukocytic count determination: WBCs were determined according to **Maxine and Benjamine (1985).**

2.2.3. Differential leukocytic count determination: Blood film was prepared according to the method of Lucky (1977). The percentage and absolute value for each type of cells were calculated according to Schalm (1986).

2.2.4. Phagocytic activity (PA) and phagocytic index (PI) determination: Phagocytic activity was determined according to Kawahara et al. (1991). The number of phagocytized candida cells was counted in the phagocytic cells to calculate the phagocytic index.

2.2.5. Lysozyme activity estimation: Serum lysozyme activity was measured with the turbidimetric method described by Engstad et al. (1992); using 0.2 mg / ml lyophilized Micrococcus lysodekticus as the substrate in phosphate buffer adjusted to pH 5.75. Fifty microlitres of serum was added to 3 ml of bacterial suspension. The 540 nm absorbance was by spectrophotometer after-mixture (Ao) and after incubation for 30 min at 37 C (A). The measured result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min. (Lysozyme activity = {(A0-A)/A}.

2.2.6. Bactericidal activity estimation: Serum bactericidal activity to Aeromonas hydrophila strain was determined according to Rainger and Rowley (1993). Briefly, a 300 µl of Aeromonas hydrophila suspension (1.5 x 10^3 cells / ml) and 300 µl of fresh serum were mixed in sterile tubes. A blank consisted of 300 µl of bacterial suspension and 300 µl of sterile PBS. The tubes were incubated at 28 °C. A 50 µl sample was removed at 0 , 1 , 2 , 3 , 4h , and different dilutions were plated on nutrient agar for 24 h at 28 C , and colony forming units (CFU) were counted . The results were recorded as survival index (SI) (Ward Law and Unlles, 1978). Values were Calculated as follows: SI = CFU at end / CFU at start x100.

2.2.7. Heamagglutination Inhibition test for detection of Newcastle antibodies: The microtechnique of haemagglutination inhibition test was done according to Takatasy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

2.3. Assessment of some blood parameters:

2.3.1. Determination of other blood pictures: RBCs and PCV were determined according to Maxine and Benjamine (1985). Hb was determined according to Lucky (1977) using commercial kits produced by Biodiagnostic..

2.3.2. Serum glucose: was determined according to Trinder (1969).

2.3.3. Serum lipids profile: serum total cholesterol, serum high-density lipoprotein (HDL), serum low-density lipoprotein (LDL) and serum Triglyceride were estimated according to Allain et al. (1974), Hopes-Virella et al. (1977), Assmann et al. (1984), and Sidney and Barnard (1973) respectively using commercial kits produced by Biodiagnostic. Serum VLDL-cholesterol and CHO/HDL ratio were calculated**.**

2.3.4. Liver function blood serum parameters: serum glutamic oxaloacetic transaminase (sGOT) and serum glutamic pyruvic transaminase (sGPT) were estimated according to Reitman and Frankel (1957) using commercial kits produced by Biodiagnostic.

2.3.5. Kidney function blood serum parameters: estimation serum uric acid and creatinine according to Fossati et al. (1980) and Giorgio (1974) respectively using kits produced by VITRO SCIENT.

2.4. Statistical analysis: Data were statistically analyzed by using the statistical analysis program (SPSS, 2017). Growth performance, feed efficiency parameters, RBCs, WBCs, total bacterial count, coliform count and lactobacillus count were transformed to the logarithmic scale before the analysis. Analysis was performed on both non-transformed and transformed data. However, results were similar in two analyses; therefore, the non-transformed data was used. Duncan's multiple range test (Duncan, 1955) has been used for multiple comparison between means at $P<0.05$ and $P<0.001$. Kolmogorov-Smirnov's test has been used to test the normal data distribution .

3. Results and discussion

3.1. Effect of different levels of lignocellulose with and without probiotic supplementation on immune respose parameters.

3.1.1. Blood picture:

The statistical analysis of our results (table 2) revealed that, lignocellulose using in broilers feed (0.5% and 1%) non-significantly (P≥0.05) elevated the WBCs count but decreased (P≥0.05) the RBCs count, Hb% and PCV% of broilers in comparison with chicks received diet without LC. Concerning probiotics supplementation with different levels of LC in broiler diet, it was noticed that the probiotics without LC inclusion increased (P≥0.05) the WBCs count while had no effect on the RBCs count, Hb% and PCV% of broilers chicks compared with the control group. Dietary combination of probiotic with LC (0.5% and 1%) resulted in non-significant (P≥0.05) elevation of RBCs count, Hb% and PCV%.

Londok and Rompis (2022) concluded that the dietary fiber (6%) in broilers diet decreased RBCs and Hb and increased TWBCs count than lower fiber level (5%). In agreement with our findings Cetin et al. (2005) concluded that the dietary probiotic addition non-significantly increased TWBCs. In the same line Fairushin et al. (2022) evaluated the influence of B.subtilis supplementation on immune response of broilers, their finding revealed that, chicken received B. subtilis had slight increase (2.11%) in the level of WBCs due to an increase in nonspecific resistance. Also Shi et al. (2020) revealed that, dietary supplementation of Bacillus subtilis in laying hen feed had no effect on RBCs and Hb concentration. Which is consistent with the results of previous studies of Park and Kim (2014) and Park et al. (2018) revealed that dietary Bacillus subtilis supplementation could not affect the concentration RBC levels in broilers. Gutierrez and Corredor (2017) and Rivera-Pérez et al. (2021) also showed that probiotics had no significant effect on the hematological values of the broilers. However, normal blood counts do not ignore the elevation of WBC count. Zhou et al. (2000) concluded that supplementation of lactic acid bacteria strains didnt affect the RBC counts or Hb.

3.1.2. Differential leucocytic counts:

The statistical analysis of the obtained results (table 3) revealed that, using dietary LC increased monocyte% (P≥0.05), heterophil% (P≥0.05) and H/L ratio (p<0.05) while it decreased lymphocyte (p<0.05), basophil (p<0.05) and eosinophil (P≥0.05) in comparison with control group. Probiotics supplementation in broilers feed elevated (P≥0.05) the heterophil % and H/L ratio but lowered in lymphocyte (p<0.05), monocyte (P≥0.05), basophil (P≥0.05) and eosinophil% (P≥0.05) compared to birds fed the same diet without probiotics supplementation. Chicks reared on diet containing LC plus probiotic increased heterophil % and H/L ratio and reduced lymphocyte and basophil% compared to groups fed on LC or probiotic separately.

Determination of differential leukocytic counts is among the simplest animal immune function measures (Norris and Evans, 2000). Heterophils and lymphocytes are the primary constitutive components of innate and adaptive immune defences, respectively (Minias, 2019). Although there is empirical proof for H/L ratios correlating positively with the strength of innate immune response (MacColl et al., 2017) and negatively with humoral adaptive response (Kerimov et al., 2018). Thus the elevated H/L ratio in our results should be associated with a state of readiness to cope with infection through innate immunity (via heterophils). In linearity with our results McReynolds et al. (2009) illustrated that insoluble fiber like alfalfa Provoked heterophils function in laying hens compared to control basal feed. In agreement with our findings de Carla Dias et al. (2020) concluded that the dietary probiotic addition significantly increased heterophil % and decreased (p<0.05) lymphocyte and monocyte. Also Cetin et al. (2005) found that the dietary probiotic addition non-significantly increased heterophil % and decreased (P≥0.05) lymphocyte, monocyte, and eosinophil%. Rivera-Pérez et al. (2021) also showed that probiotics highered neutrophils and lowered lymphocyte in broiler chickens. Darsi and Zhaghari (2021) reported that, dietary probiotic decreased H/L ratio in broiler breeders at the end of the experiment.

3.1.3. Phagocytosis, lysozome activity and bactericidal activity:

From the statistical analysis of our data (table 4) it was observed that, dietary LC addition (0.5 and 1.0%) without probiotic in broilers diet resulted in significant (p<0.05) increase in phagocytic activity and phagocytic index. The dietary 0.5% LC level without probiotic non-significant (P≥0.05) elevated bactericidal activity but decreased lysosome activity. Meanwhile, 1.0% LC in the diet increased (P≥0.05) lysosome and lowered (P≥0.05) bactericidal activity. The chicks was fed probiotic supplemented diet without LC had significant (p<0.05) increase in phagocytic activity and non-significant (P≥0.05) increase in phagocytic index

and lysosome activity while bactericidal activity showed non-significant (P≥0.05) decrease compared to birds fed on basal diet without supplementation.

These data revealed that the dietary LC and /or probiotic addition increased the phagocytic and phagocytic index which may be related to the higher heterophil%. Avian neutrophils are a key event in immune defense and are highly phagocytic granulocytes which carry potent destructive enzymes that can phagocyte and destroy invasive bacteria (Maxwell and Robertson, 1998; Ostermann et al., 2002). Hussein et al. (2017) and Hussein and Frankel (2019) also noted that dietary supplementation of layer diet with insoluble fiber as LC resulted in enhancement of phagocytosis compared to non-supplemented pullets. Similarly to our study, McReynolds et al. (2009) found that insoluble fiber like alfalfa Provoked phagocytosis in laying hens, this might be beacause of bacterial degradation of fiber into short chain fatty acids, thus support mucosal structure and small and large intestines function as well increasing beneficial bacterial count in the GIT. In agreement with our findings de Carla Dias et al. (2020) concluded that the dietary probiotic (B. subtilis) addition significantly increased phagocytic capacity and could be used as immunostimulators in aquaculture. Guo et al. (2003) and Vetvicka and Oliveira (2014) also showed that supplementing Leghorn and broiler chickens with insoluble fiber enhanced the phagocytic activity.

Probiotics have also been shown to favor the non-specific phagocytic activity of alveolar phagocytes, suggesting a systemic effect by secretion of mediators that stimulate the immune system (Cross, 2002). According to Coppola and Turnes (2004), Bacillus sp. can stimulate the immune response and can be used as immunomodulators.

3.31.4. Haemagglutination Inhibition (HI):

Effect of different levels of lignocellulose with and without probiotic supplementation on haemagglutination inhibition detection of Newcastle antibodies of broiler chickens are presented in table 5. Inclusion of LC at level (0.5% and 1%) in broilers diet had significantly increased antibody titer against Newcastle disease of broilers chicken at day 14th, 28th and non-significantly at day 42nd of broilers age compared to birds reared on LC free diet. Dietary probiotic supplementation in broilers feed non-significantly elevated the antibody titer against NDV of broilers chicken over all the feeding trial in comparison with broilers fed basal feed without probiotic. LC with probiotic supplementation had elevated level of the antibody titer against Newcastle disease in their blood at day 14th, 28th and 42nd compared to birds reared on probiotic supplemented feed without LC addition. In agreement with our finding, Sabour et al. (2019) reported that, dietary insoluble fiber (IF) inclusion (30 g/kg) in broilers feed elevated the antibody titer against Newcastle disease vaccine (NDV). The reason for this enhancement are, IF increase the acquired immunity via increasing mucin production and colonization of beneficial bacteria which increase acquired immunity. IF generate an equilibrium between commensal microflora and gut associated lymphoid tissue, which is regarded as a primary mechanism of the host against invading pathogens (Montagne et al., 2003). A study designed by Akilian (2015) showed that, 0.8% LC supplementation in broilers feed elevated the antibody titer against NDV in serum of supplemented birds than control.

Also Sikandar et al. (2017) and Sikandar et al. (2021) evaluated the efficacy of Bacillus subtilis probiotic in enhancement immune response of broilers, they observed higher (P <0.05) antibody titers against NDV in BS supplemented group on day 35 which reflects enhanced and ongoing plasma cell involvement in the production of antibodies till at least 18 days' post last antigenic exposure. In consistent with our results (Molnar et al., 2011; Shahir et al., 2014; Manafi et al., 2017, Fathi et al., 2018) deduced that, birds reared on B. subtilis supplemented feed had higher antibody titer against NDV. Another study conducted by Ebeid et al. (2021) showed that dietary 0.02% probiotics supplementation improved cell-mediated immune response (PHA-P induced proliferative response), serum IgY, and serum NDV titre significantly. Hatab et al. (2016) illustrated that, serum antibody titers against Newcastle disease virus in chicken fed basal diet supplemented with B. subtilis and E. faecium was significantly higher (P> 0.05) than those of growing layer chicks in the control group due to the immune-stimulatory effect of B. subtilis and E. faecium.

3.2. Effect of different levels of lignocellulose with and without probiotic supplementation on other serum blood biochemical parameters.

3.2.1. Blood serum glucose:

Our results statistical analysis (table 6) showed that dietary LC supplementation at levels (0.5% and 1%) non-significantly (P>0.05) increased blood glucose levels of broilers over all the feeding trials. Similarly, probiotics supplementation resulted in non-significant (P≥0.05) increase in blood glucose

levels compared to other groups. Combination between LC and probiotics resulted in elevation of glucose concentration in blood of broilers in comparison with broiler fed diets supplemented with LC only.

Table 2 . Effect of dietary lignocellulose and probiotic addition on some blood picture of broiler chickens.

Parameter	Supplementation						
	Control	+ probiotic (100g/ton)	+ 0.5% lignocellulose	0.5% ligno + probiotic	+ 1.0% lignocellulose	1.0% ligno + probiotic	P-Value
TWBCs ($x10^3/mm^3$) count	27.55±2.43	28.78±1.98	30.73±2.95	29.78±3.37	30.98±2.56	30.83±2.38	0.409
RBCs ($x10^6/mm^3$) count	1.63±0.13	1.63±0.20	1.58±0.10	1.63±0.16	1.62±0.11	1.63±0.12	0.994
Hb	8.13±0.63	8.13±1.02	7.88±0.51	8.13±0.81	8.08±0.55	8.15±0.58	0.994
PCV	26.81±2.08	26.81±3.35	26.00±1.70	26.81±2.69	26.65±1.82	26.90±1.92	0.994

Means denoted within the same row with different superscripts are significantly (P<0.05). Values are expressed as Means ± SD.

Table 3 . Effect of dietary lignocellulose and probiotic addition on differential leukocytic count of broiler chickens.

Parameter	Supplementation						
	Control	+ probiotic (100g/ton)	+0.5% lignocellulose	0.5% ligno + probiotic	+1.0% lignocellulose	1.0% ligno + probiotic	P-Value
Heterophil %	52.95±2.50	57.10±3.30	56.93±5.22	59.78±2.60	55.70±2.62	58.63±1.80	0.097
Lymphocyte%	$34.45±1.75^{a}$	$31.65±1.56^{b}$	$30.48±2.22^{bc}$	$28.33±1.25^{cd}$	$30.08±1.03^{bcd}$	$27.78±1.90^{d}$	0.001
Monocyte %	$5.50±0.84^{c}$	$5.20±2.53^{bc}$	$6.68±2.20^{abc}$	$6.65±1.61^{abc}$	$8.30±1.13^{a}$	$8.08±0.79^{ab}$	0.047
Basophil %	5.85±1.16	5.38±1.17	4.85±0.79	4.53±0.60	4.70±1.11	4.25±1.10	0.285
Eosinophil %	1.25±0.34	0.68±0.36	1.08±0.68	0.73±0.26	1.23±0.37	1.28±0.46	0.219
H/L ratio	$1.54±0.15^{c}$	$1.81±0.13^{bc}$	$1.88±0.30^{ab}$	$2.12±0.18^{a}$	$1.86±0.12^{ab}$	$2.12±0.19^{a}$	0.003

Means denoted within the same row with different superscripts are significantly (P<0.05). Values are expressed as Means ± SD.

Our result had been confirmed by Sun et al. (2021) who found that, adding different levels of eubiotic lignocellulose non-significantly increased (P > 0.05) blood glucose level in chickens as LC increased SCFAs production. According to De Vadder et al. (2014), SCFAs Butyrate activates intestinal gluconeogenesis (IGN) gene expression through a cyclic adenosine monophosphate (cAMP)-dependent mechanism, and the substrate of gluconeogenesis–propionate activates IGN gene expression via a gut–brain neural circuit involving SCFA receptors. In agreement with our findings de Carla Dias et al. (2020) concluded that the dietary probiotic addition significantly increased glucose. Also Hatab et al. (2016) reported that, laying hen fed on diet contain B. subtilis had increased serum glucose concentration compared with the control. This increase might be related to a temperate improvement in gluconeogenesis and increased lactose absorption (Das et al., 2005). While, our results are disagreement with Al-Kassie et al. (2008) who recorded reduction in serum glucose level in groups receiving probiotics as compared with the control.

3.2.2. Blood serum lipids concentrations:

The current results (table 7) indicated that Dietary inclusion of 0.5% LC without probiotic non-significantly increased concentration of cholesterol, LDL and HDL and decreased triglyceride (TG), VLDL and CHO/HDL ratio in blood of broilers.

1.0% LC without probiotic supplementation decreased all lipid profile (cholesterol, LDL, VLDL, TG and CHO/HDL) except HDL. This reduction in lipid profile with 1.0% LC was non-significant except CHO/HDL ratio. On the other hand probiotic supplementation non-significantly decreased cholesterol and LDL levels while increased the concentration of TG, HDL and VLDL, also probiotics resulted in significant decrease ($P<0.05$) in CHO/HDL ratio. Dietary probiotic supplementation with inclusion of LC (0.5% and 1.0%) decreased all serum lipid profile (significantly; cholesterol, LDL and CHO/HDL and non-significant; TG, LDL, VLDL) except increasing of HDL was observed with 0.5% LC compared with diet contained the same LC level without probiotic. Also LC with probiotic supplementation led to non-significant decrease in all lipid profile compared with diet contained probiotic without LC (control group).

Our results are in agreement with Bogusławska-Tryk et al. (2016) who demonstrated that lignocellulose at 0.5–1.0% significantly lowered serum TG, LDL and TCHOL content and slightly increased HDL in the broilers serum. The same results was observed by Sarikhan et al. (2009), Menge et al. (2012), Hassan et al. (2013), Safaa et al. (2014), Rahmatnejad and A. Saki. (2016) who concluded that, dietary LC inclusion in broilers fed lowered serum cholesterol, TG, LDL and increased HDL, this may be explained by the capacity of fiber to bind the bile lipids in the gut and the ability of lignin to bind bile acids in the intestinal lumen (Jung and Fahey, 1983), which may increase the fecal excretion of cholesterol and lower serum lipids.

Krauze et al. (2020) studied the impact of B.subtilis supplementation in drinking water of broilers, their finding are in agreement with our results, B.subtilis lowered LDL and elevated the HDL in the plasma compared to birds received control diet. Also Hatab et al. (2016), Chen et al. (2017) and Fathi et al. (2018) noted that, probiotic addition lowered serum cholesterol level. The lower serum cholesterol and LDL with bacillus supplementation may be due to either increase bile salt hydrolysis, excretion from the GIT and stimulating new bile acids biosynthesis, which is the main pathway of cholesterol catabolism and its removal from the body or inhibit hydroxymethyl glutarylcoenzyme-A reductase activity that involved in cholesterol synthesis, thereby slowing down synthesis it's from acetyl-CoA (Kivi et al., 2015). Sobczak and Kozłowski (2015) found no clear influence of Bacillus subtilis on the plasma level of TC and TAG in laying hens, but observed a marked increase in TC in the egg yolk fat. Sozcu (2019) reported that LC supplementation in broilers feed increase lactobacilli count cecal content of broilers. broilers were fed diets supplemented with Lactobacillus sporogenes probiotic had reduced serum concentration of TG, TCHOL, LDL and VLDL cholesterol fractions which may be associated with utilization of cholesterol by the probiotic bacteria for their own metabolism (Panda et al., 2006). Contrary to our results Owosibo et al. (2013) and Darsi and Zhaghari (2021) found a significant increase in serum cholesterol value with the supplementation of probiotics in the diet of broiler chickens and laying hens.

Table 4 . Effect of dietary lignocellulose and probiotic addition on phagocytosis, lysosome, and bactericidal activity of broiler chickens.

	Supplementation						
Parameter	**Control**	**+ probiotic (100g/ton)**	**+ 0.5% LC**	**0.5% LC + probiotic**	**+ 1.0% LC**	**1.0% LC + probiotic**	**P-Value**
Phagocytic activity (%)	40.65±2.22^{b}	44.75±1.52^{a}	45.75±0.98^{a}	43.73±2.20	44.03±1.31^{a}	45.50±1.68^{a}	0.006
Phagocytic index	1.50±0.13^{b}	1.54±0.14^{b}	1.78±0.08^{ab}	1.81±0.06^{ab}	2.09±0.55^{a}	1.79±0.06^{ab}	0.032
Lysosome	0.23±0.29	0.31±0.23	0.17±0.12	0.33±0.15	0.34±0.07	0.29±0.27	0.828
Bacteriocidal	77.58±28.74	69.25±22.94	81.68±14.56	67.15±14.69	64.73±6.76	71.25±26.21	0.843

Means denoted within the same row with different superscripts are significantly ($P<0.05$). Values are expressed as Means ± SD.

Table 5. Effect of dietary lignocellulose and probiotic addition on serum antibody titer log against Newcastle disease of broiler chickens.

Parameter	Supplementation						
	Control	+ probiotic (100g/ton)	+ 0.5% LC	0.5% LC + probiotic	+ 1.0% LC	1.0% LC + probiotic	P-Value
HI at 14th day old	6.00±0.00^{c}	6.75±0.50^{b}	6.00±0.82^{c}	6.75±0.50^{b}	7.00±0.00^{b}	8.00±0.00^{a}	0.001
HI at 28th day old	6.00±1.41^{c}	7.00±0.82^{abc}	8.00±0.00^{a}	7.75±0.50^{ab}	7.50±0.58^{abc}	6.25±1.71^{bc}	0.049
HI at 42nd day old	3.00±0.00	3.00±1.63	3.00±1.63	4.50±0.58	3.75±0.50	3.25±0.96	0.312

Means denoted within the same row with different superscripts are significantly (P<0.05)..Values are expressed as Means ± SD

3.2.3. Liver and Kidney functions blood serum parameters:

Our finding (table 8) reported that, 0.5% dietary lignocellulose inclusion without probiotic supplementation non-significantly (P≥0.05) improved all liver and kidney function related enzymes (decreased GOT, creatinine and uric acid) except GPT concentration was increased (P≥0.05) in blood of broiler. Higher LC content (1.0%) decreased (P≥0.05) serum liver function related enzymes (GOT and GPT) and increased (P≥0.05) kidney function related enzymes (creatinine and uric acid) levels. Probiotic supplementation without LC had no significant (P≥0.05) effect on serum liver function related enzymes concentration (increased GOT and creatinine but decreased GPT and uric acid) compared with control group. Meanwhile LC inclusion (0.5% and 1.0%) with probiotic inclusion significantly lowered serum GOP level compared with LC free diet with or without probiotic and relieved the negative effect of probiotic on the liver.

Because hepatic enzymes are released when degenerative changes in liver occur, these enzymes are accepted as indicators for the health status and functionality of the liver (Johnston, 1999). Current study are harmonious with Sozcu and Ipek (2020) results revealed that lignocellulose supplementation in both the 0.5 and 1 kg LC groups reduced hepatic enzyme levels (GOT and GPT) in laying hens at 38 week of age. Lower levels of GOT and GPT could be evidence for a hepato-protective effect of lignocellulose. A similar effect for lignocellulose in broiler nutrition was showed by Sozcu (2019). In agreement with our finding a research conducted by Hatab et al. (2016) found that, there were clear decrease in uric acid level in chicken fed on diet contain Bacillus subtilis probiotic those indicating the beneficial effect of the probiotic on the kidney function. On the other hand, Salim et al. (2011) reported that, some probiotics are able to utilize urea, uric acid and creatinine and other toxins as its nutrients for growth.

4. Conclusion

We concluded that either dietary LC or probiotic using enhanced immune response parameters (WBCs, heteophils, phagoctic activity and index, serum antibody titer log against Newcastle disease and lysozyme activity) and improved lipid profile (increased HDL and lowered cholestrol and CHO/HDL) and their combination magnified this enhancement. LC (0.5% - 1.0%) with probiotic supplemented diet revealed the best immune response parameters blood picture and lipid profile.

Table 6. Effect of dietary lignocellulose and probiotic addition on serum glucose concentration of broiler chickens.

Parameter	Supplementation						
	Control	+ probiotic (100g/ton)	+ 0.5% LC	0.5% LC + probiotic	+ 1.0% LC	1.0% LC + probiotic	P-Value
Glucose(mg/dl) at 14th day old	105.80±43.83	106.83±31.40	116.38±26.23	116.30±32.20.	105.90±37.88	105.10±30.30	0.991
Glucose(mg/dl) at 28th day old	112.70±48.08	153.48±13.85	144.53±16.18	158.00±7.20	123.83±36.30	153.63±10.10	0.137
Glucose(mg/dl) at 42nd day old	140.55±23.14	160.28±2.57	146.20±7.82	146.28±6.43	136.70±6.98	144.20±5.74	0.108

Means denoted within the same row with different superscripts are significantly (P<0.05). Values are expressed as Means ± SD.

Table 7. Effect of dietary lignocellulose and probiotic addition on serum lipid profile of broiler chickens.

Parameter	Supplementation						P-Value
	Control	+ probiotic (100g/ton)	+ 0.5% LC	0.5% LC + probiotic	+ 1.0% LC	1.0% LC + probiotic	
Cholesterol (mg/dl)	194.43±68.00[a]	153.23±25.41[ab]	201.55±41.87[a]	120.13±22.79[b]	170.73±29.90[ab]	130.90±30.02[b]	0.047
Triglyceride (mg/dl)	264.93±54.48	273.63±56.22	226.60±38.11	228.63±39.84	259.65±43.87	250.70±35.50	0.611
HDL (mg/dl)	32.25±10.14	38.98±1.26	46.03±9.67	48.28±7.60	47.30±13.72	41.58±9.63	0.239
LDL (mg/dl)	108.19±51.82[a]	59.53±33.04[ab]	110.21±51.33[a]	26.13±22.70[b]	71.50±34.81[ab]	39.19±26.29[b]	0.028
VLDL (mg/dl)	53.00±10.90	54.73±11.24	45.32±7.62	45.73±7.97	51.93±8.77	50.14±7.10	0.611
CHO/HDL ratio	6.00±1.68[a]	3.93±0.60[bc]	4.64±1.72[ab]	2.51±0.44[c]	3.93±1.53[bc]	3.24±0.83[bc]	0.018

Means denoted within the same row with different superscripts are significantly (P<0.05). Values are expressed as Means ± SD.

Table 8. Effect of dietary lignocellulose and probiotic addition on serum parameters related to liver and kidney functions of broiler chickens.

Parameter	Supplementation						P-Value
	Control	+ probiotic (100g/ton)	+ 0.5% LC	0.5% LC + probiotic	+ 1.0% LC	1.0% LC + probiotic	
Liver functions							
GOT (µ/L)	75.73±8.75[ab]	80.83±15.62[a]	68.73±4.41[abc]	71.30±2.51[abc]	66.40±5.35[bc]	59.18±8.35[c]	0.036
GPT (µ/L)	23.89±4.26	23.71±3.64	24.59±5.29	24.94±5.87	22.23±2.26	25.40±4.10	0.927
Kidney functions							
Creatinine (mg/dl)	2.33±0.42	2.50±0.07	2.22±0.56	1.92±0.49	2.45±0.52	2.60±1.12	0.687
Uric acid (mg/dl)	6.28±0.88	6.03±0.66	6.10±0.60	6.54±0.41	6.91±0.58	7.00±1.01	0.307

Means denoted within the same row with different superscripts are significantly (P<0.05). Values are expressed as Means ± SD.

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