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# Effect of using natural apple vinegar, garlic powder (Alsin) and black bean seed on the immune system and some of characteristics of the blood broilers Ross 308

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**Abstract .**The experiment was designed to study the effect of adding natural apple vinegar adding to water and garlic powder and black bean adding to the feed on the immune system and some blood characteristics of broilers Ross 308. A total of 256 chicks were randomly divided in eight group, (16 bird in each replicate) for 35 days. The first group was fed without additives (control) (T1). Second group was added apple vinegar to water 1 ml/l (T2). Third group was added garlic powder 0.3g/kg (T3). Fourth group was added black bean 4 g / kg (T4). Fifth group was added apple vinegar 1 ml/l water and garlic powder 0.3g/kg (T5). Sixth group was added apple vinegar 1 ml/l water and black bean powder 4g/kg in the feed (T6). Seventh group was added garlic powder 0.3 g/kg and black bean 4 g/kg in diet (T7). Eighth group was added 1 ml/l water, garlic powder 0.3 g/kg and black bean 4 g/kg in diet (T8). At the end of experiment 4 birds were chosen randomly from each group (2 birds / replicate) for anatomy and calculating the weight of some organs to evaluate the effectiveness of the immune system. Blood extraction was carried out to measure the level of immunity in birds against Newcastle disease and the study of some blood characteristics. Results showed significant differences (p 0.05) in thymus gland weight in favor of (T7) compared with (T1, T2, T3 and T4) while not significant with (T5, T6 and T8). Results showed no significant differences in the weight of spleen between all groups (p 0.05) and showed a significant increase (p 0.05) in the level of immunity against Newcastle disease and infectious bursal disease with the highest levels of (T5 and T7), while the lowest level of immunity against these diseases it was in (T6 and T8). The results showed a significant decrease in the level of cholesterol in (T2 and T6) compared with the other experimental groups. There was a significant increase in the level of protein in the blood in favor of (T7 and T8) compared with the other experimental groups. The blood glucose level was significantly lower in favor of the (T6) compared to the other experimental groups.

**Key words:** apple vinegar, garlic, black bean, blood characteristics

## 1. Introduction

The poultry industry is an important aspect in the provision of animal protein sources because it has a high nutritional value (Pourali et al., 2010). The modern education has been removed of the use of antibiotics in poultry feed due to its negative effects on human health due to the emergence of certain strains of microorganisms resistant to antibiotics ( Javandel et al., 2008).



In Europe 2006 the promotion of antibiotics has been identified (Garcia et al., 2007; Mansoub et al., 2011) for its negative effects on the health of birds and humans for the survival of their deposition in poultry meat and the emergence of a variety of micro-organisms resistant to the abundance of uses, so there was a need to find alternatives in poultry feed which lead to strengthen the growth in poultry by inhibiting pathological growth, balancing the intestinal microorganisms, improving the digestion factor, and enhance the absorbability of nutrients through the intestinal wall (Al-Harhi, 2002; El-Deek et al., 2003).

Apple vinegar is a natural product produced from apple fermentation, contains polyphenols, organic acids such as 3-9% of acetic acid, it is known as the main ingredient in apple vinegar, in addition to vitamins and minerals (Naziroglu et al., 2014), and has a great ability to eliminate toxic substances and harmful bacterial organisms (Romano et al., 2015; Iman et al., 2015). Organic acids family that are a good antidote to harmful bacteria (Koh et al., 2016), organic acids by reducing the number of harmful bacterial compounds present in the digestive system and reducing acid pH in the intestines lead to increased degradation of mineral elements such as phosphorus and increase the degradation of amino acids and fats. This is a result of increasing the effectiveness of digestive enzymes and increasing absorption capacity (Da Silva et al., 2013), and it is lead to reduce blood lipids (Beheshti et al., 2012) and improve immunity (Pourmozaffar et al., 2017).

Studies have shown that many medicinal plants are important in feeding poultry due to their vital role and the diversity of their effects (Gerson et al., 2009). The benefits of the active substances and their effect on the enzymatic activity of the gastrointestinal tract, thus improving the digestion of nutrients (Jang, 2006), such as garlic (*Allium sativum*) and its active ingredient are alicin which has a reduced effect of cholesterol and (LDL) low-density lipoprotein (<https://www.researchgate.net/publication/287719484> 2009), (AL-Shammari, 2010) a blood sugar reducer (Rizwan et al., 2011) and it is an immunostimulant for infectious diseases in poultry (Alkashta et al., 2016).

Black seeds have excellent potential as an alternative to antibiotics and vaccines to improve immunity and reduce mortality in poultry (Nasir et al., 2010). The black bean seeds contain more than 30% fixed oil and 0.4-0.45% (w/w) volatile oil, including (Ebru et al., 2008) 4-24% thymoquinone (TQ) and 46% of many monoterpenes such as *r*-cymene and *a*-piene (Al-jassir, 1992) and (Fahmy IR et al., 1958). Studies have shown that black bean has a reduced effect of cholesterol and raises HDL (AL-Beitawi et al., 2009). Active ingredients in black bean seeds possess anti-bacterial, anti-oxidant and inflammatory properties that stimulate the positive effect on the immune system and its organs (Al-Saleh et al., 2006; Arslan, 2005). (Al-Beitawi NA et al., 2009) found that the concentration of antibodies to Newcastle disease and fabrycia gland inflammation had significantly improved when replacing the seeds of the black bean replaced one of the antibiotics such as bacitracin methylene disalicylate in poultry feed. And that the studies available for vinegar and these medicinal plants and their effects on the chickens are available in a single study aimed at the current effect of the use of natural apple vinegar in water and garlic importers (Alicin) and black bean and mixtures on the immune system and some of the characteristics of the puppies of the Ross breed meat.

The aim of study was estimated effect of using natural apple vinegar, garlic powder and black bean seed on the immune system and some of characteristics of the blood broilers Ross 308

## 2. Materials and methods

The experiment was performed with a broiler chicks Ross-308 reared at the poultry research farm of the University of Al-Furat Al-Awsat Technical. They were reared in under optimal conditions from first until sixth weeks of age. Chicks were fed a starter diet (corn, wheat, soya diet), (table 1), the feed and water were offered *ad libitum* throughout all experiment period. The chicks at the first day were weighed and divided equally into eight groups of 16 birds with 2 replicates in each group. The first group was fed basal diet and pure water control (T1), the second group was fed basal diet with added 1 ml/l water of natural apple vinegar (T2). Third group was added 0.3 g/kg garlic powder in feed (T3). Forth group was added 4 g/kg black bean seeds as powder in feed (T4), fifth group was

added apple vinegar 1 ml/l water and garlic powder 0.3g/kg (T5). Sixth group was added apple vinegar 1 ml/l water and black bean powder 4g/ kg in the feed (T6). Seventh group was added garlic powder 0.3 g/kg and black bean 4 g/kg in diet (T7). Eighth group was added 1 ml/l water, garlic powder 0.3 g/kg and black bean 4 g/kg in diet (T8). The birds were vaccinated the infectious bursal disease (IBD) on the ninth day of age and the Newcastle vaccine (ND) in two doses on seventh and nineteenth of the age through drinking water to study the immunological effect of feed additives on domesticated birds. At the end of the breeding period, blood samples were collected by random selection of birds (2 birds / replicate) and drag from the vein under the wing (Al-Daraji et al., 2008). Samples were taken after the drawing into the laboratory and samples were placed in the centrifuge and by 4000 cycles / minute to separate the plasma from the serum and put the samples in the fridge under the temperature -20 for use in blood tests to measure glucose, cholesterol and total protein and used the kits produced by AGApe and the measurement process was carried out according to the recommended instructions of the manufacturer. The spectrophotometer was used to measure the level of glucose, cholesterol and total protein in the blood. The level of immunity in birds was measured at the veterinary hospital in Babel province after the blood was withdrawn immediately.

Statistical analysis was performed using (SAS 2012).

**Table 1.** Standard feed ingredients used in feeding and calculated chemical composition

<b>Ratio</b>		
	<b>Materials</b>	<b>Starter % Grower %</b>
	<b>Yellow corn</b>	40 47
	<b>Wheat</b>	20.5 21
	<b>Soybeans</b>	26 21
	<b>Animal protein</b>	10 8
	<b>Oil</b>	3 2.5
	<b>Food salt</b>	0.3 0.3
	<b>Premix</b>	0.2 0.2
	<b>Total</b>	100 100
	<b>Calculated chemical composition</b>	
	<b>Crude protein</b>	22.3 19.4
	<b>(Energy (kg / kg feed)</b>	3000 3135
	<b>Calcium</b>	1.27 1.1
	<b>Phosphorus is available</b>	0.4 0.4
	<b>Lysin</b>	1.16 1.25
	<b>Methionine</b>	0.45 0.4

### 3. Results and discussion

The results in the table 2 showed the effect of addition of natural apple vinegar, garlic extract and black bean seed powder on the weight of the immune glands of the meat breeds, indicated a significant increase in the weight of the thymus gland and fabricia gland in favor seventh group compared with other experimental groups, which did not have any significant differences except the second group, which showed insignificant decrease in the weight of the thymus gland(P 0.05). The results of the study did not show any significant differences in weight of spleen between all experimental groups.

**Table 2.** Effect of adding natural apple vinegar, garlic extract and black bean seeds on the weight of the immune glands (g) broilers.

Groups	Thymus gland	Fabricia gland	Spleen
T1	b 8.34	b 4.60	a 2.42
	1.48 ±	1.05 ±	0.24 ±
T2	c 4.06	b 3.73	a 3.65
	0.90 ±	1.42 ±	0.05 ±
T3	b 9.67	b 3.81	a 2.16
	1.45 ±	1.03 ±	0.39 ±
T4	b 8.50	b 2.30	a 3.40
	0.34 ±	0.04 ±	0.26 ±
T5	ab 9.88	b 5.04	a 3.63
	0.25 ±	0.76 ±	0.52 ±
T6	ab 9.74	b 3.46	a 2.33
	0.04 ±	0.31 ±	0.50 ±
T7	a 12.31	a 8.67	a 3.14
	0.65 ±	0.20 ±	0.35 ±
T8	ab 10.84	b 5.41	a 3.00
	0.11 ±	1.5 ±	0.92 ±

The different characters vertically (average) and within the box (overlaps) indicate significant differences between the traits at the probability level (P 0.05)

Table 3 showed the results of the additions used in this study on the immune level of broiler chicken against Newcastle disease and Infectious bursal disease. The results of the statistical analysis revealed a high level of immunity against Newcastle disease in the fifth group and the high level of immune in seventh group against the fifth-degree infectious bursal disease compared with rest experimental groups . Table 4 shows a significant decrease in the level of glucose in serum (P 0.05) in favor of the sixth group compared with all other experimental groups except the seventh group were not significant which it followed by seventh group compared with the rest of the transactions except the fifth group was not significant effect. The highest level of glucose was observed in the control group.

**Table 3.** Effect of adding natural apple vinegar, garlic extract and black bean seeds at the level of immunity against Newcastle Disease (ND)and Infectious Bursal Disease (IBD) in broiler

Groups	ND	IBD
T1x	abc 2385.0	ab 1732.0
	15.00 ±	92.00 ±
T2	abcd 2320.0	ab 1657.5
	40.00 ±	87.50 ±
T3	bcd 2215.0	ab 1615.0
	135.00 ±	125.00 ±
T4	abcd 2320.0	ab 1697.5
	30.00 ±	12.50 ±

<b>T5</b>	a	a
	2562.5 137.00 ±	1801.0 119.00 ±
<b>T6</b>	d	b
	2062.5 2.50 ±	1492.5 2.50 ±
<b>T7</b>	ab	a
	2485.0 25.00 ±	1807.5 2.50 ±
<b>T8</b>	cd	ab
	2170.0 70.00 ±	1595.0 55.00 ±

The different characters vertically (average) and within the box (overlaps) indicate significant differences between the traits at the probability level (P 0.05)

Table 4 showed a significant decrease in serum cholesterol level (P 0.05) in second group and in sixth group Where they were 100.17 and 102.47, respectively, compared with the rest of transactions. The highest level of cholesterol was in the third group where it was 151.78, as for the total protein level in the blood serum there was a significant increase in favor of the seventh and eighth groups respectively (P 0.05) compared to the other groups followed by the second, sixth, first and third, respectively and there were not significant effect between them but were significant with the fourth and fifth group, that contained the lowest level of protein in blood serum.

**Table 4.** Effect of adding natural apple vinegar, garlic extract and black bean seeds on the blood characteristics of broiler chicken

Groups	Glucose	Cholesterol	Total protein
<b>T1</b>	a	ab	b
	213.0± 3.00	139.5± 11.27	6.00± 0.6
<b>T2</b>	a	c	b
	205.52± 1.95	100.17± 0.13	6.45± 0.15
<b>T3</b>	a	a	b
	206.90± 1.72	151.78± 1.03	5.55± 0.3
<b>T4</b>	a	b	c
	208.93± 0.38	123.61± 3.50	4.05± 0.35
<b>T5</b>	ab	b	c
	200.86± 0.86	128.15± 6.92	3.0± 0.30
<b>T6</b>	c	c	b
	180.16± 2.58	102.47± 1.24	6.15± 0.35
<b>T7</b>	bc	ab	a
	187.06± 6.38	137.74± 4.16	8.10± 0.30
<b>T8</b>	a	ab	a
	206.03± 9.48	140.56± 2.00	7.95± 0.55

The different characters vertically (average) and within the box (overlaps) indicate significant differences between the traits at the probability level (P 0.05)

The significant decrease in glucose in the sixth treatment (apple cider vinegar and black bean seed powder) is due to the containment of the black bean on thymoconin which is considered the main active ingredient in the seeds of the black bean which plays an important role in lowering blood sugar

(Al-hialy and Hamed 2004). The results of this study correlate with the results of Karmous et al. (2016) who found the effect of active compounds in black bean powder such as thymocoquine and hydrothymoconion in reducing blood sugar in broilers fed on diets containing different proportions of black bean powder.

Apple cider vinegar which is contain in components on 3-9% acid (Beheshti et al., 2012) is one of the organic acids known to be used as an antibacterial agent in the field of food industry (Koh et al., 2016). Most organic acids reduce the number of bacterial groups in the digestive system and increase acidity of the gastrointestinal tract which increases the level of melting or disintegration of nutrients, minerals and fat in the stomach and digestive tract, which increases the efficiency of the digestive system and thus improves the absorbability of materials. Food and its transmission through blood to tissues and cells of the body (Da Saliva et al., 2013), This is also evidence of the effectiveness of organic acids to reduce the level of cholesterol, as shown in the results shown in Table 4 of the low level of cholesterol in the treatment of the second treatment and the sixth (P 0.05), The results of this study were consistent with those of(Tollba and Hassan, 2003; Hassan et al., 2007; Al-Beitawi et al. 2009), confirming in the results of their studies that the seeds of the black bean reduce the level cholesterol in serum. Low cholesterol levels in the serum may be due to the high content of unsaturated fatty acids found in black seeds, which may induce cholesterol into the intestines and oxidation (Khodary et al., 1996).

The high level of total protein in serum in the seventh and eighth group depends on the availability of protein in the diet of birds as the protein found in the powder of black bean seeds was available to birds that contained in diet them on this material (Khan et al., 2012), as well as the effect of apple cider vinegar in improving the internal environment of the digestive system which leads to increased susceptibility to absorption, in addition to the effect of garlic powder in the improvement of growth and feed conversion ratio (Tollba and Hassan, 2003).

It was conclude that addition of natural apple vinegar in the drinking water and garlic powder (Alsin) and black bean seed in feed led up to improve immunity system and have act against some diseases such as Newcastle disease and infectious bursal disease and have positive effect on blood characteristics of Ross 308 broilers.

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