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RESEARCH ARTICLE

Effects of Soybean Meal Processing Method on the Broiler Immune System [1][2]

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Summary

Various methods of processing soybean meal were evaluated for their effects on the immune system parameters of broilers in a completely randomized design. The experiment was carried out in 200 male Ross broilers, grouped as quadruple. Soybean meal in the chicken feed was prepared by five different processes; i.e. raw soybean meal, autoclaved (121°C) for 20 min, autoclaved for 30 min, microwaved at 46°C, 540 watt for 7 min and baked at 120°C for 20 min. The serum antibody titers of broiler vaccinated against infectious bronchitis was determined at 23 days that against Newcastle was done at 27 days, that against Gumboro was done at 30 days and that against sheep red blood cells was done at 28 and 42 days. Results showed that baked soybean meal could help promoting the broiler serum antibody titers against various vaccines as well as against sheep red blood cells at 28 days ($P<0.05$).

Keywords: *Soybean Meal, Processing, Immune, Vaccines, Antibodies*

Soya Küspesi İşleme Metodunun Broyler İmmun Sistemine Etkisi

Özet

Çalışmada tamamen tesadüfi tasarı içinde farklı soya küspesi işleme metodlarının broylerlerde immun sistem parametreleri üzerine etkisi incelendi. Deney dörtlü olarak gruplandırılmış 200 erkek Ross broylerlerde gerçekleştirildi. Tavuk rasyonunda soya küspesi, ham soya küspesi, 20 dakika otoklavlanmış (121°C'de), 30 dakika otoklavlanmış, 46°C'de mikrodalgada fırında tutulmuş, 540 watt'ta 7 dakika tutulmuş ve 120°C'de 20 dakika pişirilmiş olmak üzere 5 farklı işlem ile hazırlandı. Serum antikor titreleri infeksiyöz bronşite karşı aşılanmış broilerde 23. günde, Newcastle'a karşı aşılananlarda 27. günde, Gumboro'ya karşı aşılananlarda 30. günde ve koyun eritrositleri inokule edilenlerde 28. ve 42. günlerde belirlendi. Sonuçlar pişirilmiş soya küspesinin broylerlerde çeşitli aşıların uygulanması ve koyun eritrositleri inokulasyonu (28. günde) sonucunda serum antikor titrelerini arttırdığını göstermiştir.

Anahtar sözcükler: *Soya küspesi, İşleme, Bağışıklık, Aşılar, Antikorlar*

INTRODUCTION

With rapid population growth, the supply of animal protein needs is important. Given the characteristics of poultry meat, the production system and the possible return on investment in a shorter time and other features, attention and further study is necessary in the field of poultry, especially broiler chickens. Academic research and scientific and technical progress achieved in recent decades, poultry industry is reach to the degree of progress that is considered among the world's economic arteries and also since the industry plays an important role in providing animal protein for humans, so there have been a special place in the global economy. Share in the global economy is subject to remove local needs and produce competitive products. Achieving this goal requires being equipped with the latest findings in the field of poultry science and creates a lasting and dynamic relationship between academic centers and manufacturing [1].

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Soybean meal is a food used in poultry. Soybean meal is better than other protein sources due to the growing cost of this product, high quality soy protein, the high rate of energy metabolism (2540) high protein (44-48) and the relative balance of amino acids (in comparison with other vegetable protein contains more lysine) [2]. Soybean meal is contains a number of toxins, irritants and inhibitors include allergens, goitrogens and anti-clotting factors. Protease inhibitors are of special importance in nutrition, antitrypsin and the inhibitor Bowman-Birk chymotrypsin have practical significance [3]. The mechanism of anti-nutritional factors in the body is somewhat similar. Some of these effects are: non-digestible starch and protein, availability of mineral extraction, development of anemia and reducing the growth [4,5]. Inhibitors are inactivated by heating, so in order to having satisfactory nutritional quality, soybean meal needs to heat process in the poultry nutrition. Aburt et al.[6] stated that from a practical standpoint, the process is the most important factor affecting the digestibility of amino acids, because most of animal powders and vegetable meal which use as a protein source in broiler chicken rations are influenced by temperature. Both high and low temperatures are effective on amino acid digestibility of food, but the higher pressure and temperature are probably more effective [7]. Digestibility of amino acids decreases due to the heat because heat reduces the amino acid conformation and concentration, and other parts are because of the Millard reaction between the essential amino acids, particularly lysine with oligosaccharides such as raffinose and stachyose. Vegetable meal, in particular, soybean meal, containing high amounts of these oligosaccharides [7].

Since very little research has been conducted around the world on the effect of processed soybean meal on immune system of broilers, the present study is designed to investigate the effect of processing on the mentioned factors.

MATERIAL and METHODS

Experimental Animals

Two hundred of one-day-old Ross broilers were used. The experiment was carried out quadrupled in a completely randomized design with five types of processed soybean meal. The average weight of the chickens was 42 g.

Broiler Chicken Rearing

The research was carried out in the brooder houses and the Milk Industry Animal Nutrition Laboratory, Faculty of Agriculture in Islamic Azad University at Rasht branch. The hall was divided using metal divisions into 1.5×1.5 m experimental units. Ventilation system was supplied using window fans and air conditioner. Hall lighting was supplied by 100 watt light bulb and the room temperature using the central heat supply system.

The rearing house was disinfected by fumigation with formaldehyde gas for 24 h. The drinkers for broiler were filled with sugar water. The room temperature was warmed up using electric heater to a level of 32-33°C. Then, the temperature was gradually reduced to 22°C in day 27th and maintained until the end of the experimental period. Lighting for the hall on the first day was 24 h and from the second day to the end of the experiment was manipulated as a "23 hour-light and 1-hour-dark" cycle.

Soybean Meal Processing Method

Soybean meal was prepared as 1.5 kg per batch. For the autoclaving process, the soybean meal was autoclaved (121°C and 1 Pascal pressure) using Iran Teb Zaeem autoclave ^{2000} for 20 min and 30 min, respectively. Then, the samples were removed and transferred to a tray to cool down prior to transferring to plastic bags and were kept at proper temperature.

In the case of baking, this was conducted in feed and milk industries Laboratory Faculty of Agriculture, Islamic Azad University, Rasht branch using Do 636 Memert Oven, UNB400 model. In order to reach an even temperature, soybean meal was spread out in an aluminum tray to obtain an evenly 2 cm-thick layer and placed in an oven set at 120°C for 20 min. After thermal curing, the samples were removed and transferred to a tray to cool down prior to transferring to plastic bags and were kept at proper temperature.

For the microwave processing, the soybean meal was conducted in feed and milk industries Laboratory Faculty of Agriculture, Islamic Azad University, Rasht branch (LG microwave, 540 watts, TCR 4284-CC, Korean). Before moisture processing, soybean meal was measured by the psychrometer and brought to 25% moisture content. Then, soybean meal was placed in a 5-7 cm diameter Pyrex plate and microwaved for 7 min. Prior to transferring to a tray to cool down prior to transferring to plastic bags and were kept at proper temperature.

Chicken Feed Composition and Nutrient Value

The composition of used diet and nutrient composition of diets in this period was demonstrated in *Tables 1* and *2*, respectively. Rearing period as indicated were shown; i.e. starter period (1-14 days), grower period (15-35 days) and finisher period (36-42 days).

Brand Names of Vaccines Used

In this experiment, it was used Nobilis® Gumboro 228E for vaccination against Gumboro**,** Nobilis® IB 4/91 for the immunization of chickens against infectious bronchitis, and NOBILIS ND LASOTA for the immunization of chickens against Newcastle disease.

Birds Treated with Different Vaccines and SRBC

It was vaccinated 4 broilers per treatment (1 bird per replication) and totally it was vaccinated 20 broiler per

Table 1. *Used diets during experimental periods*
Tablo 1. *Deneme süresince kullanılan rasyonlar*

Ingredient (%)	Starter	Grower	Finisher
Corn	46.09	50.91	48.88
Fish meal	3.00	3.00	-
Meat meal	3.00	3.00	-
Oil	4.56	5.45	7.39
Soybean meal	40.00	35.00	39.97
DL-Methionine	0.29	0.23	0.17
L-Lysine*HCL	0.04	-	-
L-Threonine	0.03	-	-
Ca%22P%18	0.99	0.75	1.64
CaCO3	0.98	0.76	1.00
K-Bicarbonate	0.05	-	-
NaCl	0.37	0.37	0.45
KCl	-	0.03	-
Vitamin and Mineral mixture	0.60	0.50	0.50
Total (%)	100	100	100

Table 2. *Nutrients analysis of used diets during experimental periods*
Tablo 2. *Deneme dönemlerinde kullanılan rasyonlarda analiz edilen besinler*

Ingredient	Starter	Grower	Finisher
Dry Matter (%)	89.54	89.54	89.86
Crude Protein	24.90	23.0	22.0
Energy (ME) (kcal/kg)	3025	3150	3200
Lysine (SID) (%)	1.27	1.13	1.10
Methionine (SID) (%)	0.36	0.55	0.47
Met+Cys (SID) (%)	0.94	0.84	0.76
Threonine (SID) (%)	0.83	0.74	0.72
Tryptophan (SID) (%)	0.26	0.24	0.24
Arginine (SID) (%)	1.55	1.41	1.40
Iso-Leucine (SID) (%)	0.93	0.85	0.84
Valine(SID) (%)	1.02	0.94	0.91
Leucine(SID) (%)	1.79	1.68	1.64
Calcium (%)	1.05	0.90	0.85
Available Phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.23	0.23	0.20
Potassium (%)	1.00	0.90	0.93
Chloride (%)	0.30	0.30	0.30
DCAB (mEq/kg)	272.12	244.55	242.77
Choline (g/kg)	1.48	1.37	1.37
Linoleic Acid (%)	1.21	1.27	1.24
Ether Extract (%)	6.84	7.87	9.22
Crude Fiber (%)	3.78	3.52	3.73
Lysine (Total) (%)	1.41	1.26	1.22
Metionine (Total) (%)	0.67	0.59	0.50
Met+Cys (Total) (%)	1.05	0.94	0.85
Threonine (Total) (%)	0.98	0.87	0.85
Tryptophan (Total) (%)	0.30	0.27	0.28
Arginine (Total) (%)	1.68	1.54	1.51
Iso-Leucine (Total) (%)	1.04	0.95	0.94
Valine (Total) (%)	1.16	1.07	1.03
Leucine (Total) (%)	1.99	1.87	1.82
Lysine (TFD) (%)	1.26	1.12	1.09
Metionine (TFD) (%)	0.64	0.56	0.47
Met+Cys (TFD) (%)	0.95	0.85	0.77
Threonine (TFD) (%)	0.86	0.77	0.75
Tryptophan (TFD) (%)	0.26	0.23	0.24
Arginine (TFD) (%)	1.54	1.40	1.38
Iso-Leucine (TFD) (%)	0.95	0.86	0.86
Leucine (TFD)(%)	1.83	1.72	1.68

vaccination step. As there are 3 vaccinations and also 2 SRBC injections, thus 100 broilers were administrated under vaccines and SRBC.

Vaccination

In this experiment, three types of vaccines were used, which were administered as two injection methods to the broilers. Bronchitis vaccine was done at day 1 and 16 of age, Newcastle vaccine was at day 8 and 20 of age, and Gumboro vaccine was injected on day 14 and 23 of age.

Utilization of the watering systems found in poultry housing is a common method to administer live vaccines. Birds were restricted water prior to vaccinating in order to make the birds thirsty enough to ensure all birds are ready to drink once the vaccine is administered. Water consumption is an important variable to calculate so that the correct amount of water can be used to mix with the vaccine. For houses without a water meter, a practice run using only water two days before vaccination will verify the amount of water needed [8].

Prior to vaccination, all medication, disinfectants and chlorine were removed from the drinking water 72 h before vaccination. Administer the vaccine in the water early in the morning. Sufficient drinker space is prepared to allow free access to the vaccine solution [8].

When preparing vaccine, prepared the vaccine directly in the water tank. The addition of skim milk powder to the water 20-30 min prior to adding the vaccine is applied as a stabilizer. It is added the skim milk powder at the rate of 500 g/200 liters. Afterwards, it is opened the vaccine vial by removing the aluminum seal and the rubber stopper. Using the water that used in the vaccination, it is filled the vial approximately two thirds full. Then it is closed the vial with the rubber stopper and gently shake in order to reconstitute the lyophilized vaccine. It is rinsed the vaccine vials several times to remove all the vaccine. It is poured the reconstituted vaccine into the drinkers [8].

The first vaccine used in this study was the bronchitis

vaccine which was applied at the first day of rearing chickens before sending them to a pen. In this case, 2.000 doses of bronchitis vaccine were mixed with the amount of 500 ml of distilled water and pour into a spray device to spray on the broilers at the distance of 50 cm.

Other vaccines were given in a drinking water. Since the vaccine should not be left out for too long, the broilers were made to be thirsty by keeping them at 1-2°C increased temperature for 2 h (6:00 to 8:00 am) prior to placing the drinkers containing limited amount of water mixed with vaccine. The vaccine mixed water had to be all consumed before supplying more fresh water into the drinkers.

The doses of bronchitis vaccine were given at the age of 16 days and 23 days. One bird from each pen was randomly selected and 1 ml of blood was taken from the veins in its wings. Then syringes were placed tilted at the angle of 30 degree in order to separate blood cells from the serum. The blood samples were transferred to the laboratory for evaluation of bronchitis titers.

Newcastle (B1) vaccine was given to broiler at the age of 28 days through drinking water. One week after that, one bird from each pen was randomly selected and 1 ml of blood was taken. After separation of blood cells from the serum, the blood samples were transferred to the laboratory for evaluation of Newcastle titers.

Gumboro vaccine was given in the drinking water at the age of 14 and 21 days of age and the blood samples were taken using the same process from the 30-day-old bird.

Injection of Sheep Red Blood Cells (SRBC)

The evaluation of humoral response to the bird's body against to sheep red blood cells were carried out from two injections to the 22- and 35-day-old birds and blood samples were taken at the day 28 and 42, respectively, for immune response evaluation.

The injection of sheep red blood cells to the 22 and 35 day-old broilers, 30 ml of sheep red blood cells (SRBC) were mixed with samples in phosphate buffered saline (PBS). Then, 0.5 ml of the solution was randomly injected in two per experimental unit into the birds breast muscle and six days after the first injection and seven days after the second injection of sheep red blood cells in the age of 28 and 42-days, 1 cc of blood taken through the veins of its wings. Then syringes were placed tilted at the angle of 30 degree in order to separate blood cells from the serum prior to transferring to the laboratory.

Determination of Antibody Titration in Infectious Bronchitis Virus (IBV) and Gumboro (Infectious Bursal Disease, IBD)

For determination of antibody titer in IBD and to measure IBV antibody titer, one bird from each replicate were selected. The blood after about 2 h was placed at room temperature under 45 degree angle to be separated from its serum. The samples are then transferred to the laboratory. In this study, the ELISA method using commercial kits Bio check to determine antibody titer was used for IBV and IBD.

ELISA which was derived from the first letters of Enzyme Linked Immuno Sorbent Assay has been defined for the first time in 1971. In the rapid experiment for the separation and determination of antibody titer, antigen is used against the virus or bacteria and other materials. In this method, enzymes were used for marking antigens or antibodies and enzyme levels were measured with ELISA Reader device. Temperature of 20-25°C was applied for testing. Increase or decrease in temperature will cause changes in test.

HI Test (Haemagglutination Inhibition Test)

Blood sample was taken from one bird. HI test was used to determine vaccine titers of ND. HI tests are based for virus or bacteria ability for agglutination red blood cells. In this experiment, the antigen located in the presence of the studied serum and red blood cells. If the serum contains special antibodies and antigen binding, antigen was neutralized and lost the ability of agglutination red blood cells. So, the agglutination inhibition (HI) reaction occurs.

Sheep Red Blood Cell (SRBC)

Birds from each replicate were injected 0.1 ml per kg of body weight by 0.5% sheep red blood cells into the wing vein. Blood samples were taken. First, after serum separation and decomplementation in 56°C the HA tests were done. Twent-five µl serum and 25 µl phosphate buffer saline (pbs) added into the first 96 well plates (8×12) and the plates were incubated at 37°C for half an hour. After half an hour to rest wells (pbs) 25 µl was added. And then dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 were prepared. After preparation this dilution 25 µl SRBC (1 percent solution) added to each well, then the plates incubated for 45 min at 37°C and then the number of first slipping cell was recorded. Titers were reported based on $\log_2$.

Since IgM is sensitive to 2-Mercaptoethanol (2Me) and it will be destroyed in its presence by adding this material. After this, the observed titer represents a variant of the IgG. SRBC titer difference is obtained from total IgG titer, IgM titer. For measuring antibody sensitive to (2Me), 25 µl serums with 0.01M (pbs) was incubated at 37°C for half an hour. Other steps are like testing of total anti-SRBC. Antibody resistant to 2- Mercaptoethanol IgG were obtained from the fraction of the total anti-SRBC titers.

Data Analysis

All collected raw data was entered into the Excel software and after categorization was conducted in a randomized

complete block design. Data were analyzed by SPSS statistical software, and averages were compared by multi domain Tukey testing and were compared statistically at 5%.

Before performing the analysis of variance, Normality test was carried out, and, if necessary, data were used for transformation. The statistical model was as follows:

$X_{ij} = \mu + a_j + e_{ij}$

μ = is the average of the samples was evaluated through null hypothesis

X_{ij} = The observed value

a_j = The effects of experimental diets

e_{ij} = Experimental error for each observation

RESULTS

The results of these experiments are summarized in *Table 3*.

Antibody Titers Against Infectious Bronchitis Virus Vaccine in 23rd Days

The results from comparison of methods for processing soybean meal showed that processing had no significant effect on antibody titers against infectious bronchitis virus vaccine in blood of broilers (P>0.05). Comparison of the averages obtained in numerical experiments showed that treatment of oven had the greatest impact on antibody titers against infectious bronchitis virus vaccine in blood of broilers. However, this difference was not statistically significant (P>0.05) and followed by control, autoclaved 20 min and microwave and autoclaved 30 treatment showed the weakest effect on antibody titers against infectious bronchitis virus vaccine in blood of broilers parameter.

Antibody Titers Against Newcastle Virus Vaccine in 27th Days

The results from comparison of methods for processing soybean meal showed that processing had no significant effect on antibody titers against Newcastle virus vaccine in blood of broilers (P>0.05). Comparison of the averages obtained in numerical experiments showed that treatment of oven had the greatest impact on antibody titers against Newcastle virus vaccine in blood of broilers. However, this difference was not statistically significant (P>0.05) and followed by control, autoclaved 20 min and autoclaved 30 min and microwave treatment showed the weakest effect on antibody titers against Newcastle virus vaccine in blood of broilers parameter.

Antibody Titers Against Gumboro Virus Vaccine in 30th Days

The results from comparison of methods for processing soybean meal showed that processing had no significant effect on antibody titers against Gumboro virus vaccine in blood of broilers (P>0.05). Comparison of the averages obtained in numerical experiments showed that treatment of oven had the greatest impact on antibody titers against Gumboro virus vaccine in blood of broilers. However, this difference was not statistically significant (P>0.05) and followed by microwave, control and autoclaved 30 min and autoclaved 20 min treatment showed the weakest effect on antibody titers against Gumboro virus vaccine in blood of broilers parameter.

Antibody Titers Against Sheep Red Blood Cells in 28th Days (First Injection)

The results from comparison of methods for processing soybean meal showed that processing had significant effect on antibody titers against sheep red blood cells in blood of broilers (P<0.05). Comparison of the averages showed

Table 3. *Mean comparison (±SEM) of immune parameters in broiler blood among five studied tratments **
Tablo 3. *Çalışılan beş uygulama arasında broiler kan immün parametrelerin ortalama karşılaştırması (±SEM) **

Trait / Treatment	IMMUNE SYSTEM RESPONSE				
	Against SRBC (First Injection) [log2]	Against SRBC (Second injection) [log2]	Against Gumboro Vaccine [log10]	Against Infectious Bronchitis Vaccine [log10]	Against Newcastle Vaccine [log2]
1 (Control)	3.250±0.250 [b]	3.500±0.289 [a]	5074.250±1498.574 [a]	1398.500±1122.692 [a]	4.750±0.479 [a]
2 (Autoclaved soybean meal: 121°C, 20 min)	3.500±0.500 [ab]	4.000±0.408 [a]	4004.000±1505.175 [a]	907.750±161.118 [a]	4.500±0.289 [a]
3 (Autoclaved soybean meal: 121°C, 30 min)	4.000±0.408 [ab]	5.000±0.577 [a]	4287.750±1350.292 [a]	251.500±124.824 [a]	4.250±0.250 [a]
4 (Browned soybean meal: 120°C, 20 min)	5.000±0.408 [a]	5.250±0.479 [a]	6446.000±268.827 [a]	1489.250±561.624 [a]	5.000±0.408 [a]
5 (Macrowaved soybean meal: 46°C, 540 Watt, 7 min)	4.250±0.250 [ab]	4.500±0.289 [a]	5895.000±1703.304 [a]	566.750±139.079 [a]	4.000±0.577 [a]
CV (%)	18.82	19.02	53.07	124.00	18.59

** Means in each column followed by the same letters are not significantly different at α=0.05*

that oven treatment had the greatest impact on antibody titers against sheep red blood cells in blood of broilers ($P<0.05$) and followed by microwave, autoclaved 30 min and autoclaved 20 min and control treatment showed the weakest effect on antibody titers against sheep red blood cells in blood of broilers parameter. Also, microwave, autoclaved 30 min and autoclaved 20 min was no significant difference with each other and control, autoclaved 20 min, autoclaved 30 min and microwave treatment were in a same level and autoclaved 20 min, autoclaved 30 min, oven and microwave were in the same statistical level.

Antibody Titers Against Sheep Red Blood Cells in 42nd Days (Second Injection)

The results from comparison of methods for processing soybean meal showed that processing had no significant effect on antibody titers against sheep red blood cells in blood of broilers ($P>0.05$). Comparison of the averages obtained in numerical experiments showed that treatment of oven had the greatest impact on antibody titers against sheep red blood cells in blood of broilers. However, this difference was not statistically significant ($P>0.05$) and followed by autoclaved 30 min, microwave, autoclaved 20 min treatment and control treatment showed the weakest effect on antibody titers against sheep red blood cells in blood of broilers parameter.

DISCUSSION

Broiler chickens due to the potential for rapid growth have high susceptibility to infectious agents [9]. And in this regard is clear that nutrition play significant role in regulating and modulating the immune function and response in broiler chickens [10].

By stimulating the immune system by foreign proteins it can observe antibodies against this protein. The power of this antibody is used as an indicator of the ability of the humoral system in animal immunology and ecology research [11]. The immune response based on genetic variation and environmental variation, which also contains the feedingr factor, will be vary [12].

A strong response indicating more power of host against foreign pathogens so response of obtained antibody has positively correlated with host general resistance against disease [13]. Data obtained from this experiment showed that, processing soybean meal in broiler chickens has led to an increased immune response.

Hamilton and Sandstedt [14] have reported that in identical diets with regard to nitrogen which is part or all of them have been replaced with browned soybean meal affected levels of browned soybeans, weight gain, and feed intake and feed conversion ratio, so that increasing levels of browned soybeans were significantly decreased feed conversion and feed consumption ($P<0.05$).

Also Waldroup and Cotton [15] reported that with consumption of 20% processed soybeans; the result is better performance, so that with consumption of processed soybean, energy level of feed intake is more. The performance of browned soybeans is always better than raw soybeans.

Kaankuka et al.[16] stated that consumption of raw soybeans in poultry rations, increase the weight of the pancreas gland (eight percent versus 37 percent of live weight), duodenal weight (1.35 vs 1.6% of live weight), and reduced food intake and growth chickens. When soybean oil is extracted what are remains is meal which is about 48% protein, 35-40% carbohydrates, and 7-10% water, 5-6% minerals and less than 1% fat [17].

As Ladics [18] stated the production of antigen-specific antibodies represents a major defense mechanism of humoral immune responses. Several assays have been developed to assess T-cell-dependent antibody responses (TDAR). Of these assays, the antibody forming cell assay (AFC) or plaque forming cell (PFC) assay and ELISA are the two most often used tests to assess immunotoxicity. Historically, the T-cell-dependent antigen of choice has been sheep red blood cells (SRBC). The SRBC AFC assay is considered the gold standard for TDAR based on extensive intra- and inter-laboratory validation [18].

Studies have shown that anti-nutritional factor in plants and their seeds are because of insecticide and resistant pests of this material. Because these materials are kept grain against to attack mold, bacteria, insects and birds [19-21].

Due to the large variety of cereal grains can not be precisely defined the anti-nutritional factors more present in the shell or endosperm**,** but in general, according to the experiment most anti-nutritional factors present in whole grains is more than shell or endosperm alone [22]. The mechanism of anti-nutritional factors in the body is somewhat similar. The most important effects of anti-nutritive factors in the body in short can be so named as: Changes in pancreatic size, reduced protein digestion, agglutinated red blood cells, abnormal uptake in the intestinal mucosa, Metabolism of bile acids and cholesterol reduction, removal of available mineral elements, reducing the absorption of nutrients, inactivated the respiratory chain enzymes, causing bloating and digestive discomfort [4,5,15,23,24].

Saponins in poultry diets can reduce food intake, have a bitter tasteand reduced food intake, not only because of the bitter taste but due to mouth and digestive tract damage by the saponin, and can irritate mucous membrane of the intestine and cause to various biological effects such as increased excretion of cholesterol and prevent of growth. These compounds are not destroyed by heat [25-27]. Soybeans containing 0.5% of the saponin and its three times consumption has no effect on performance of poultry. Because these materials hydrolysis by bacteria enzyme of lower small intestine [28].

Raw soybeans contain weak substances goiter. One of the components of goitrogens is one oligopeptide, two-three amino acids, or a glicopeptid. Add a small amount of iodine in the diet will result in the loss of its effects [28,29].

Raw soybeans contain three percent of the hem-agglutinin. And their ability to clot blood cells in different animal species is different. And a small part of the growth of broiler chicks and mice has been attributed to the hem-agglutinin [19].

Lectin is one of the raw soy protein inhibitors [20,21,30]. Lectin is easily capable of binding with the sugars. Effect of anti-nutritional composition of lectin is because of its adhesion with the intestinal mucosa which damage intestine lining cells [31-34].

Douglas et al.[35] reported that broiler that had received diets containing soybean without the Lectin compared to broilers consumed of diets containing raw soybeans had significantly higher growth performance.

Lectin increased intestine activity and as a result enlarge intestine. In general, the main effects of lectin are include decreased significantly in the lectin digest and absorb nutrients and endogenous nitrogen loss is due to increased cell shell in the gastrointestinal tract. Lectins in the cell wall thickening of small intestine and colon that prevents absorption of nutrients. Most of the toxic lectin effect is because of lectin entry into the blood circulatory system in the body and producing antibodies. The possibility also exists that poisoning resulting from the use of lectin is due to the dominance of a group of bacteria in the digestive tract.

One of the major factors that lead to inhibition of lectin is the ability of them against enzyme protease [34,36-38]. Lectin soybean can act as a growth factor pancreatic, so that is no effective on insulin only its secretion back to the pancreas exocrine [39]. Protease inhibitors are also increased pancreatic weight that this effect had been observed in mice that had been taking kidney bean [40,41].

Since most of the enzymes secreted from the pancreas of sulphur and sulphur-rich amino acids .Their loss is caused by deficiency of methionine. Thus, inhibitors of legume will reduce protein in the diet and loss of androgenic protein [42]. Also trypsinogen and chymotrypsin enzymes are containing cysteine values which is an amino acid that is rich in sulphur. The disposal of large amounts of enzymes imbalance amino acid. Also the defective protein degradation and excreted reduce nitrogen absorbed [43].

Perform appropriate processing, is useful for increasing the digestibility of amino acids. One disnature factors of food proteins the heat processing. One method is to autoclave curing temperature. In this method, heating with steam under pressure is used. Disnature of protein depends on heat intensity and duration of heating [44].

Roasting is a process that is rooted in prehistory. But the roast somewhat modified by the industry to meet the needs of today. There are several models that are including the same types of heating systems commonly used for grain drying and wet heating system. Heat by the oven can be used to burning coal or be produced directly by a flame. Produced temperatures between 110 and 170°C due to the equipment used are variable [45].

Thomason [46] suggested that he outlet temperature of seeds should be between 110 to 113°C the for monogastric and for mammals is about 116°C to increase the protein content that can be analyzed by the mammalian. This process reduces the moisture content of grain up to thirty percent but without degradation of cellular structures or release their oil, so most of the grains must be milled before use as food or plates. Different methods are used for making browned that the key difference between these methods is a situation in which heat is used (dry or wet). In each case the same method should be implemented, so the situation is avoided in which some components of crude remained, the outer layer of another material than can be processed.

The microwave method is relatively new and the waves with wavelengths between 0.1 to hundreds of cm. Energy spread by the waves by polar water molecules are absorbed into the grains, so producing friction and heats the grain [47].

Hafez et al.[48] studied long-term effects of nutritional factors on grain processing and production parameters of broiler chickens. Nine minutes of processing time to produce the best results, while Millard reaction time increases, higher and lower indices reversed the positive effects provided.

Yoshida and Kajimoto [49], proved that 2450MH microwave processing in order to reduce anti-nutritional factors in soy to acceptable levels, to require 24 percent humidity.

Antibody titers against the studied vaccine showed that thermal processing significantly increased titer antibody response against sheep red blood cells transfusions in 28 - days and had the most effect on browning soybean meal. In terms of numerical processing to improve the immune response against the vaccine bronchitis, Gumboro and Newcastle, in all cases, the browned soybean meal has the most effect on improving the immune system.

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