African Journal of Microbiology Research Vol.6 (26), pp. 5464-5471, 12 July, 2012
Available online http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1594
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Temperature treatment of soybean meal on intestinal microbial flora in broilers

Mojgan Nahavandinejad[1], Alireza Seidavi[1]* and Leila Asadpour[2]

[1]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran.
[2]Department of Veterinary Medicine, Rasht Branch, Islamic Azad University, Rasht, Iran.

Accepted 24 January, 2012

The experiment was carried out to evaluate the effects of different temperature treatments of soybean meal on broiler microflora in a completely randomized design with five treatments and four replications and a total of 200 strains of Ross rooster. In this experiment, the broilers were fed diets containing raw soybean meal, autoclaved soybean meal (121°C, 20 min), autoclaved soybean meal (121°C, 30 min), microwaved soybean meal (46°C, 540 Watt, 7 min) and browned soybean meal (120°C, 20 min). The results obtained showed that the temperature treatments improved the cecum microbial population, and the soybean meal (autoclave 20 min) significantly led to increased cecum lactic acid bacteria population of broiler (P<0.01). The results of the comparison of temperature treatments of soybean meal showed that processing did not affect the population of total bacteria, *Escherichia coli*, Coliforms bacteria, and Lactobacillus bacteria in the cecum of broiler chickens (P>0.05).

Key words: Soybean meal, broiler, process, microbial population, intestine.

INTRODUCTION

In the poultry industry, the cost mainly spent on nutrition and the poultry industry is currently witnessing a dramatic increase in price of the main constituent of poultry feed such as corn, soybean meal, fat and poultry byproducts in the global market. Like other livestock industries, the poultry industry is always looking for ways to exploit the market in order to minimize feed cost, and any improvements and progress in this area can reduce the cost and in turn bring more economical benefit (Sheppard, 2004).

Protein in poultry diets is the most important factor affecting food production and the performance of poultry. Protein in poultry diets are provided from a large amount of plant protein sources (Scott et al., 1982). Protein sources after energy supply sources composed most of the poultry diets. Due to the increase in demand for vegetable protein sources in poultry nutrition, productivity of all available protein sources should be examined. Quality sources of protein are functions of the amino acid composition, its digestibility and the presence of anti-nutritional substances in them (Destar et al., 2004).

Autoclave and microwave are considered the most important heating methods that may play a role to destroy anti-nutritional compound especially trypsin inhibitor, phytic acid, lectin, and tannins. One of the factors of protein denaturation is thermal processing. In autoclave, the heat was used with steam under pressure. Protein denaturation under heat depends on the intensity and duration of heating (Roder et al., 1999). With regard to the price of soybean meal and its use in broiler diets, this meal will be allocated a large part of the total cost of production. In general, most research has focused on the idea that the proper temperature should be seen for maximum protein quality. The aim of all reprocessing practices is increasing the nutritional value of food. Each method of processing is associated with some advantages and disadvantages. Selecting the appropriate method of these methods requires a comparison of this process in the same conditions. However, very little research has been conducted around the world in regard to the processing of soybean meal on microflora of broilers. On the other hand, the untreated SBM contains

*Corresponding author: E-mail: alirezaseidavi@iaurasht.ac.ir.

various anti-nutritional factors that negatively affect the digestive functions, but these factors can be reduced by thermal treatment but causes gelatinization which affects the nutrient availability of monogastric (Friedmen et al., 1991; Marsam et al., 1997; Perrilla et al., 1997; Sakomura et al., 1998). This study aimed to investigate the effect of processing on the factors listed earlier.

MATERIALS AND METHODS

Time and space experiments

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. This experiment was conducted in 2011 for 42 days. Inside the hall, metal divisions were divided into 1.5×1.5 m experimental broiler per division.

Ventilation system was supplied using window fans and room air. Hall lighting was supplied by 100 watt light bulb and the room temperature using the central heat supply system.

Preparation of brooder houses

First, the litter, manure, and all non-fixed equipment such as drinkers and feeders were removed out of the hall and room, and its equipment was thoroughly washed with water. The floor was burned with a flamethrower and floor and walls of the hall was sprayed with lime and all experimental units were flat, in the bed was a cardboard roll. Preoperative gasification with formaldehyde gas, all doors and windows of the hall pores were closed; all equipment was placed in the hall. Twenty four hours after the gasification operation, all doors and windows were kept open and fans for better exhaust gas was switched. Before the arrival of the broiler, drinkers for broiler were filled with sugar water and room temperature using the heater reached from 32 to 33°C.

Rearing management

Two hundred and one-day old Ross broiler rooster strain were purchased and transferred to the experiment site. The experiment took place in a completely randomized design with five treatments and four replicates. Raising chickens in the first day, after accidentally weighing between treatments and repetitions of each experiment were divided; so that in each experiment, there were 10 broilers and their average weight was (42±1.52 g).

Room temperature from 31°C on the first day, was gradually reduced to 22°C in day 27 and to the end of the growing period, the temperature was maintained. Lighting for the hall on the first day was 24 h and from the second day until the end of the period was 23 h light and 1 h off day. To maintain sanitary conditions in the hall, disinfectant solution for cleaning up the pond in the entrance hall was thrown, which was replaced daily. Vaccinations and generally, other health care operations was administrated in the region and directed by the veterinarian responsible.

The studied treatments

In this study, five treatments were evaluated: (1) soybean meal (control), (2) autoclaved soybean meal (121°C, 20 min), (3) autoclaved soybean meal (121°C, 30 min), (4) browned soybean meal (120°C, 20 min), and (5) microwaved soybean meal (46°C, 540 Watt, 7 min).

Control soybean meal

Soybean meal used in broiler diets without any temperature processing.

Autoclaved soybean meal (121°C, 20 and 30 min)

Autoclaving of the soybean meal was conducted in feed and milk industries, Laboratory Faculty of Agriculture, Islamic Azad University, Rasht branch using Iran Teb Zaeem autoclave ^{2000}. In this case, each time 1.5 kg of soybean meal was placed in the autoclave for 20 and 30 min at 121°C and 1 Pascal pressure. After treatment, the samples were removed and transferred to a tray and allowed to cool down. After cooling, the meal was transferred to plastic bags and kept at proper temperature.

Browned soybean meal (120°C, 20 min)

The browning of soybean meal 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 temperature for the entire meal equally, meal was poured to a special aluminum container by height of 2 cm and at a constant temperature of the oven at 120°C were placed for 20 min inside the oven. After thermal curing, the samples were removed and transferred into the tray to be cool. After cooling, meal was transferred to plastic bags and was kept at proper temperature.

Microwaved soybean meal (46°C, 540 Watt, 7 min)

Microwaving the soybean meal was conducted in feed and milk industries, Laboratory Faculty of Agriculture, Islamic Azad University, Rasht branch using household LG microwave, TCR 4284-CC, Made in Korean. Before moisture processing, soybean meal was measured by the psychrometer and then soybean meal was brought to 25% moisture, then soybean meal was placed within 5 to 7 cm diameter Pyrex for 7 min in the 540 watts microwave. After the meal was exposed in the microwave, the samples were removed and transferred into the tray to be cool. After cooling, meal was transferred to plastic bags and was kept at proper temperature.

Research stage and periods

Since the end of the broiler rearing period, three experiments were used: starter period (1 to 14 days), grower period (15 to 35 days), and finisher period (36 to 42 days).

Used diets

The composition of used diet and nutrient composition of diets in these periods are shown in Tables 1 and 2.

Preparing the medium

The medium was prepared for this experiment to allow bacteria growth and to count the number of colonies. One day before sampling, microbial cultures were obtained in Laboratory Animal Feed and Dairy Industries, Faculty of Agricultural Sciences, Islamic Azad University, Rasht Branch.

Preparing Eosin methylene blue (EMB) agar medium

For the preparation of EMB agar, according to the suggested

Table 1. Used diets during experimental periods.

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
$CaCO_3$	0.98	0.76	1.00
K-Bicarbonate	0.05	-	-
NaCl	0.37	0.37	0.45
KCl	-	0.03	-
Vit and Min.Mixture	0.60	0.50	0.50
Total (%)	100	100	100

agenda for preparing the medium, an amount of powdered substrate (36 g per liter) was mixed with distilled water in erlenmeyer flask, and then placed on a hot plate until clear solution was obtained. Then the obtained solution was placed inside the autoclave for twenty minutes at 121℃; after sterili zation, the solution was left to cool. After reaching a temperature of 45℃ in liquid medium under the hood and side burner, the solution was poured on the rigid medium prepared in the plates.

Preparing McConkey medium

For the preparation of McConkey agar, according to the suggested agenda for preparing the medium, an amount of powdered substrate (50 g per liter) was mixed with distilled water in Erlenmeyer flask, and then placed on a hot plate until clear solution was obtained. Then the solution was placed inside the autoclave for twenty minutes at 121℃; after sterilization, the s olution was left to cool. After reaching a temperature of 45℃ in liquid m edium under the hood and side burner, the solution was poured on the rigid medium prepared in the plates.

Preparing Rogassa medium

For the preparation of Rogassa agar, according to the suggested agenda for preparing the medium, an amount of powdered substrate (74.5 g per liter) was mixed with distilled water in erlenmeyer flask, and then placed on a hot plate until clear solution was obtained. Then the solution was placed inside the autoclave for twenty minutes at 121℃; after sterilization, the s olution was left to cool. After reaching a temperature of 45℃ in liquid m edium under the hood and side burner, the solution was poured on the rigid medium prepared in the plates.

Preparing MRS medium

For the preparation of MRS agar, according to the suggested agenda for preparing the medium, an amount of powdered substrate (68.2 g per liter) was mixed with distilled water in erlenmeyer flask and then placed on a hot plate until clear solution was obtained. Then the solution was placed inside the autoclave for twenty minutes at 121℃; after sterilization, the so lution was left to cool. After reaching a temperature of 45℃ in liquid medium under the hood and side burner, the solution was poured on the rigid medium prepared in the plates.

Preparing nutrient agar medium

For the preparation of Nutrient agar, according to the suggested agenda for preparing the medium, an amount of powdered substrate (20 g per liter) was mixed with distilled water in Erlenmeyer flask and then placed on a hot plate until clear solution was obtained. Then the solution was placed inside the autoclave for twenty minutes at 121℃; after sterilization, the so lution was left to cool. After reaching a temperature of 45℃ in liquid medium under the hood and side burner, the solution was poured on the rigid medium prepared in the plates.

Preparing pepton water (dilution method)

The solution was prepared for the dilution of samples from cecum. In order to prepare pepton water, according to the suggested agenda for preparing the medium, an amount of powdered substrate (25.5 grams per liter) was mixed with distilled water in Erlenmeyer flask and then 9 cl was poured in each tube; after which the tubes were placed inside the sterilized autoclave for twenty minutes at 121℃.

Microbial tests

At the age of 39 days, birds from each replicate (four birds per treatment) were randomly selected and slaughtered. After separation of the cecum, one gram of cecum content was removed from the bird. To determine the colony forming units (CFU), one gram of the cecum contents in the vicinity of a hot flame was added to 9 ml pepton water (tube number one). The solution was properly shaken. Then, 1 ml of solution was added to the next tube (tube number two) by a sampler containing 9 ml of sterile pepton water. This operation was done until tube number eight and a dilution series was prepared.

Dilution of 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} of the samples was performed,

Table 2. Nutrients analysis of used diets during experimental periods.

Nutrients	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

and the samples were placed on a plate containing eosin methylene blue agar, McConkey agar, Rogasa medium, and MRS agar for growth of bacteria *Escherichia coli*, growth of coliforms, growth of lactobacillus bacteria, and for lactic acid bacteria growth; however, nutrient agar medium was prepared for counting the total cultured bacteria. This process was repeated for each sample.

Culture McConkey, eosin methylene blue and nutrient agar at 37℃ for 24 h and Rogasa and MRS for 72 h in anaerobic jar was incubated at 37℃. Finally, the samples between 25 to 300 colonies, which can be counted was selected as an appropriate dilution, and after being counted it was multiplied by their inverse dilution and the number of bacteria was obtained.

Statistical analysis

During the research, all collected raw data were entered into the Excel software, and after categorization, it was conducted in a randomized complete block design.

Data were analyzed by SPSS statistical software and the averages were statistically compared by multi domain Tukey testing at 5%. Before performing the analysis of variance, Normality test was carried out, and if necessary, data were used for transformation. The statistical model is as follows:

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

Table 3. Mean comparison (±SEM) of cecum microflora among five studied treatments*.

Trait treatment	Total bacteria [cfu/gr]	*Escherichia coli* [cfu/gr]	Coliforms bacteria [cfu/gr]	Lactobacillus bacteria [cfu/gr]	Lactic acid bacteria [cfu/gr]
1 (Control)	$8.23\times10^{8a}\pm1.804\times10^{8}$	$43376433.33^{a}\pm2381570.292$	$76752533.33^{a}\pm1.010\times10^{7}$	$1.22\times10^{8a}\pm2.392\times10^{7}$	$2.18\times10^{8b}\pm0.693\times10^{7}$
2 (Autoclaved Soybean Meal: 121℃, 20 min)	$5.93\times10^{8a}\pm6.178\times10^{7}$	$22325733.33^{a}\pm6451889.300$	$48433333.33^{a}\pm2.009\times10^{7}$	$1.34\times10^{8a}\pm2.959\times10^{7}$	$3.26\times10^{8a}\pm1.415\times10^{7}$
3 (Autoclaved Soybean Meal: 121℃, 30 min)	$6.34\times10^{8a}\pm5.846\times10^{7}$	$22175733.33^{a}\pm1631014.675$	$43666666.67^{a}\pm1.099\times10^{7}$	$1.21\times10^{8a}\pm3.483\times10^{7}$	$3.35\times10^{8a}\pm2.021\times10^{7}$
4 (Browned Soybean Meal: 120℃, 20 min)	$7.40\times10^{8a}\pm1.501\times10^{8}$	$35751166.67^{a}\pm144342.282$	$33051100.00^{a}\times2.857\times10^{7}$	$1.99\times10^{8a}\pm2.646\times10^{7}$	$3.31\times10^{8a}\pm3.435\times10^{7}$
5 (Macrowaved Soybean Meal: 46℃, 540 Watt, 7 min)	$8.47\times10^{8a}\pm4.060\times10^{8}$	$29833333.33^{a}\pm7779960.011$	$53366666.67^{a}\pm1.039\times10^{7}$	$2.00\times10^{8a}\pm1.436\times10^{7}$	$3.24\times10^{8a}\pm1.456\times10^{7}$
CV (%)	3.842×10^{13}	2.653×10^{12}	5.076×10^{16}	1.255×10^{14}	3.194×10^{14}

*Means in each column followed by the same letters are not significantly different at P>0.05.

$\mu_{=}$ is the average of the samples 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 this experiment are summarized in Table 3.

Microbial count of aerobic total bacteria

Comparison of the study's methods for processing soybean meal showed that processing has no significant effect on the cecum total bacteria of the broiler chickens (P>0.05).

When the data obtained were compared, it was observed that autoclave treatment for twenty minutes statistically had the highest effect on the total bacteria population in the cecum of broiler chicken; although this difference was not statistically significant (P>0.05), it was followed by autoclave for 30 min, oven and control, and finally, microwave had the lowest effect on total bacteria of broiler cecum.

Microbial count of *Escherichia coli* bacteria

Comparison of the study's methods for processing soybean meal showed that processing has no significant effect on the *Escherichia coli* bacteria of the broiler cecum (P>0.05). When the data obtained were compared, it was observed that autoclave treatment for 30 min statistically had the highest effect on the *E. coli* bacteria population in the cecum of broiler chicken; although this difference was not statistically significant (P> 0.05), it was followed by autoclave for 20 min, microwave and oven, and finally, the control had the lowest effect.

Microbial count of coliform bacteria of cecum contents

Comparison of the study's methods for processing soybean meal showed that processing has no significant effect on the coliform bacteria of the broiler cecum (P>0.05). When the data obtained were compared, it was observed that oven treatment statistically had the highest effect on the coliform bacteria of cecum population in the cecum of broiler chicken; although this difference was not statistically significant (P>0.05), it was followed by autoclave for 30 min, autoclave for 20 min and microwave, and finally, the control had the lowest effect on the coliform bacteria of broiler cecum.

Microbial count of lactobacillus of cecum contents

Comparison of the study's methods for processing soybean meal showed that processing has no significant effect on Lactobacillus of cecum contents of the broiler cecum (P>0.05). When the data obtained were compared, it was observed that data microwave treatment statistically had the highest effect on the lactobaci llus of cecum contents population in the cecum of broiler

chicken; although this difference was not statistically significant (P>0.05), it was followed by oven, autoclave for 20 min and control, and finally, autoclave for 30 min had the lowest effect on the lactobacillus of cecum contents of broiler cecum.

Microbial count of lactic acid bacteria of cecum contents

Comparison of the study's methods for processing soybean meal showed that processing had statistically significant effect on lactic acid bacteria of cecum contents of the broiler cecum (P<0.01). When the data obtained were compared, it was observed that autoclave for 30 min treatment statistically had the highest effect on the lactic acid bacteria of cecum contents population in the cecum of broiler chicken (P<0.01), followed by oven, autoclave for 20 min and microwave, and finally, the control had the lowest effect on the lactic acid bacteria of cecum contents of broiler cecum. More so, control treatment is in one level and autoclave for 30 min, oven, autoclave for 20 min and microwave are in the same statistical level.

DISCUSSION

Microbial population of the gastrointestinal tract plays an important role on the performance of bird health (Javed et al., 2002). The change of number or types of bacteria can affect the immune system or the physiology of the digestive system; bacteria may compete for nutrients or the release nutrient feeds with the host. Most absorption of birds nutrients are carried out in the small intestine. Due to the increase in bacteria, a competition between microbes and host occurs in the feed intake. Bacteria in the colon affected by fermentation of undigested nutrients produced volatile fatty acids; but due to the limited absorption, the nutrients in this part of the digestive tract are low. However, the bacteria that are not fixed depend on the host status. In addition, bacteria can alter food and reduce the ability to use them for poultry (Bedford and Schulze, 1998). The results of this study indicate that treatments receiving processed soybean meal had the highest populations of bacteria producing lactic acid, while various methods of processing soybean meal had no effect on microbial population of coliform bacteria, *E. coli*, lactobacillus, and total microbial population.

Currently, there is no available information about the processing of soybean meal on intestinal bacterial populations in broiler chickens. This research has shown that medicinal plants can stimulate the growth of immune organs (Hevener et al., 1999; Takahashi et al., 2000). Soy seed contains protein, fat, carbohydrate and mineral elements. Protein and fat include more of the commercial value of soy. These compounds are located mainly in the cotyledons. Amounts of protein and seed oil are due to climate change, and genetic differences in soil properties are between 30 and 46% (Chorn, 2000), and are 12 to 24%. The amino acid found in soybeans are from 5.93 to 6.19 (sixteen grams per gram of nitrogen), while the sulfur amino acid are from 1.18 to 71.1 (sixteen grams per gram of nitrogen) is variable (Pourreza, 1991; Latifi, 1993).

Soybean meal is a good source of amino acid. However, due to the high consumption of this meal in poultry feed, there is need to pay more attention to its quality (Swick, 2001). The uncommon and limiting amino acid for growth is sulphur amino acid (Reddy, 1996). Different varieties of soybean meal have no important differences in sulfur amino acids.

Soybean meal protein in comparison with other plant proteins has more lysine (Swick, 2001; Wiryawan and Dingle, 1998) and is considered as a good source of arginine, glycine, and tryptophan, though two amino acid, methionine and cystine are poor (Robert, 2001; McNab and Boorman, 2002). Methionine is the first limiting amino acid in the very energetic diet and is very important. A large amount of soy in the diet should be used from methionine and methionine-containing foods as a supplement. Protease inhibitor in soybeans has been widely studied. Most of these enzyme inhibitors are effective inhibitors of the trypsin and another group is effective group of chymotrypsin, kunitz inhibitor has an active position in which the composition is irreversible with trypsin. Bowman birk inhibitor has two different positions. One binding position with trypsin and a binding position with chymotrypsin and are prevented from the activity of trypsin and can affect digestion of proteins. Due to some anti-nutritional factors like tannins, trypsin inhibitor and lectins, especially in immature seeds, grain digestibility and intake by animals are reduced (Jansman et al., 1993).

Anti-nutritional factors in monogastric animals caused growth inhibition, reduced feed efficiency, goiter, enlargement of the pancreas and the liver is damaged (liener, 2000). Consumption of raw soybean caused to create anti-nutritional complications such as enlargement of the pancreas and reduction of fat absorption (NRC, 1994). The presence of inhibitors, low levels of proteases in the intestine cannot disable the peptides; therefore, these peptides bind to the intestinal lining and stimulate cells secreted into the intestine can not be stopped for a long time. The final result of stimulation and secretion of pancreatic enzymes and intestine is a part of proteins secreted by the pancreas into the feces that comes out of the cycle and can not be reconstructed (Owyang et al., 1986).

Boiling in water, autoclave and microwave are the most important heating methods that can play important role to destroy anti-nutritional compounds, especially trypsin inhibitor, phytic acid, lectin and tannin (Habiba, 2002; Vijiykumari et al., 1998). The use of thermal treatments

on meals caused the inactivation of inhibiting growth (Zhang et al., 1993), trypsin inhibitors, Lectins, goitrogens and anti-vitamins (Arba and Dale, 1990; Lazaro et al., 2006). Heat causes the compound to absorb metihionine-cystine and the animal is able to use it. This material should not be overly influenced by temperature (Farkhovi and Sanei, 1998). Increasing temperature and heating time because of the reduced size of some amino acids can be used (Schneeman et al., 1977).

In general, the process is to be completed correctly for both systems to produce similar results. Although various studies that were conducted during the nineties in this area showed that, digestibility of amino acids in the soybean meal by a single stomach is better than soy. The availability of fats in soybean free oil was high when in the oil seeds. Herkelman et al. (1992) observed that heating soybean reduced urease index and anti-trypsin factors, and improved apparent digestibility of nitrogen and essential amino acids.

Gundel and Matrai (1996) compared digestibility of various nutrients in raw processed grains, soybean meal and oil. All the various methods (Cooking and lamination, roasting, microwaving and dry extrusion) presented digestibility values, energy, protein and better lysine seeds than the type of the mixture of meal and oil. The data obtained by the university of Nottingham and Madrid with the process used were associated by the energy value of soybean (Mateos et al., 1996).

In another experiment, Piers et al. (1997) reported that heating healthy cottonseed increase the amount of milk production to the rate of 5%. They also stated that healthy cotton mill and then heating it, increases the 0.1% fat milk. Mabjeesh et al. (1998) reported that heating the fiber increased decomposition of cottonseed; these results have been obtained in almost all experiments that used heated cottonseed. However, the manner, amount and duration of heating varied between experiments. In another experiment, Mabjeesh et al. (2000) reported that heating healthy cotton seed due to high digestion of processed cotton seed carbohydrates leads to further increase in the rumen digestion of fat.

Bernard and Calhoun (1997) reported that there was no significant difference between the fat of cows fed diets containing cottonseed heated healthy firm, but plating and putting pressure on cottonseed reduced milk fat percentage.

Arieli et al. (1989) reported that insoluble nitrogen content in acid detergent insoluble nitrogen (ADIN) in healthy cotton seed and cotton seed heated in oven at 140℃ for two hours were similar.

In the process of heating cottonseed, Wedegaertner and Lalor (1997) stated that excessive heat (greater than 140℃) reduced the availability of lysine and prote in digestibility in the small intestine. At high temperatures, browning reaction caused to create some compounds which can not be used in the small intestine.

Soybean processing methods used for their energy and protein content increases, although the exact method used should depend on processing conditions and species for food. Digestibility of energy increased because of the energy function according to the release of proteins and lipids in cells. In both cases, the type of processing is less and it could damage a part of the protein. The first part of the anti-nutritional factors was destroyed and this reduces the use of amino acids. In cases where processing is excessive, some reaction occurs between amino groups of free amino acids and sugars in the seeds (Millard reaction) decreased part of the protein. These two effects occur together and are inevitable. In other words, while the heat is used, anti-nutritional factors are destroyed; Millard reactions are produced by the reduced availability of amino acids. For mammals, a small amount of additional heat will increase the protein content that can not be digested by them, although the final outcome will be generally useful for the production of dairy cows. For monogastric, those methods used for the browning will be less intracellular fat free. More Millard reactions were produced than the variety of methods based on moist heat, therefore, the amount of energy and protein digestibility of the grain improved to a lesser extent.

Conclusion

Microflora of broiler cecum was affected by thermal processing and it improved the population of beneficial bacteria in the cecum of chickens. It has a significant effect on the population of lactic acid bacteria which increased intestinal blind, though autoclaved soybean meal had the greatest effect on bacterial. Thermal processing of soybean meal resulted in the reduction of total cecum bacteria and reduced the population of coliforms and *E. coli* bacteria, but this reduction was not significant. Overall, the autoclave and browning has the best effect on their population.

ACKNOWLEDGMENTS

This manuscript was obtained from the MSc thesis of Mojgan Nahavandinejad at Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch, Rasht, Iran for their support.

REFERENCES

Arba M, Dale N (1990). Evaluation of Protein solubility as an indicator of over processing of soybean meal. Poult. Sci. 69:76-83.

Arieli A, Ben Moshe A, Zarnwel S, Togari H (1989). *In situ* evaluation of the ruminal and intestinal digestibility of heat-treated whole cottonseed. J. Dairy Sci. 72:1228-1288.

Bedford MR, Schulze H. (1998). Exogenous enzymes for pig and poultry. Nutr. Res. Rev. 11:91-114.

Bernard JK, Calhoun MC (1997). Responses of lactating dairy cows to mechanically processed whole cottonseed. J. Dairy Sci. 80:2062-

2068.
Chorn L (2000). Use of soybean meal and oil in diets of sea bream and sea bass. Available in: www. Asa.europe.org/pdf/sbmibdiets.pdf.
Destar B, Golian A, Eftekhar Shahroudi F, Kermanshahi H. (2004). Determining digestible lysine and sulfur amino acid requirements of broilers in the first stage of growth. J. Agric. Sci. Nat. Res. 8(4):99-109.
Farkhovi M, Sanei MB (1998). The role of protein in poultry nutrition. Education Research Unit, Department of Agriculture, p. 215
Gundel J, Matrai T (1996). Different methods of heat treatment for soybeans in piglet nutrition. 2nd International Full-fat Soya Conference. American Soybean Association. Budapest, Hungary, pp. 433-450.
Habiba RA (2002). Changes in antinutrients, protein solubility, digestability and HCL extrability of ash and phosphorus in vegetable peas as affected by cooking methods. Food Chem. 77:187-192.
Herkelman KL, Cromwell GL, Stahly TS, Pfeiffer TW, Knabe DA (1992). Apparent digestibility of amino acids in raw and heated conventional and low trypsin inhibitor soybeans for pigs. J. Anim. Sci. 70:818-826.
Hevener W, Routh PA, Almond GW (1999). Effects of immune challenge on concentrations of serum insulin-like growth factor-1 and growth performance in pigs. J. Can. Vet. 40:782-786.
Jansman AJM, Verstegen MWA, Huisman J (1993). Effects of dietary inclusion of faba beans (Vicia faba L.) with a low and high content of condensed tannins on digestion and some physiological parameters in piglets. Anim. Feed Sci. Tech. 43: 239-257.
Javed MT, Sarwar MA, Kausar R, Ahmad I (2002). Effects of feeding different levels of formalin (37% formaldehyde) and urea on broiler health and performance. Veterinarski Arch. 72:285-302.
Latifi N (1993). Soybean farming: Agriculture - Physiology - uses. University Press, Mashhad Ferdosi university.
Lazaro R, Mateos GG, Latorre MA, Piquar J (2006). Whole soybeans in diets for poultry. American Soybean Association.
Liener IE (2000). Non-nutritive factors and bioactive compounds in soy. Page: 13-45 in soy in animal nutrition. J.K. Drackley ed.fed. Animal Science Sac., Savoy, IL.
Mabjeesh SJ, Arieli A, Zamwell S, Tagari H (1998). Heat-treated whole cottonseed versus maize gluten meal as a rumen undergradable protein supplement for lactating cows. Live. Prod. Sci. 55:249-259.
Mabjeesh SJ, Galindez J, Kroli O, Arieli A (2000). The effect of roasting whole cottonseed on milk production by dairy cows. J. Dairy Sci. 83: 2557-2563.
Mateos GG, García P, Medel P (1996). The use of full- fat soybeans in diets for poultry. En: 2nd International Full-fat Soya Conference. American Soybean Association. Budapest, Hungary, pp. 324-337.
McNab JM, Boorman KN (2002). Poultry feedstuffs, supply, composition and nutritive value". CABI Puplication, pp. 221-235.
National Research Council (NRC) (1994). Nutrient requirement of poultry. 9th rev.ed National Academy press, Washington, DC. p. 155.
Owyang C, Louuie DS, Tatum D (1986). Feedback regulation of pancreatic enzyme secration. Suupression of cholecystokinin release by trypsin". J. Clin. Invest. 77:2042-2047.
Piers AV, Eastridge, ML, Firkins JL, Lin, YC (1997). Effects of heat treatment and physical processing of cottonseed on nutrient digestibility and production performance by lactating cows. J. Dairy Sci. 80:1685-1694.
Pourreza J (1991). Scientific principles and practical poultry rearing. Publications of Jihad University , Isfahan Industrial University. p. 322.
Reddy CV (1996). Soybean meal in poultry diets. Poult. Int. 35(5):44-49.
Robert AS (2001). An update on soybean meal quality considerations. American soybean Assocation.
Roder H, Sauder JM, Park SH (1999). Kinetic and Structural Characterization of Early Even Events In Protein Folding. In: Old and New Views of Protein Folding, Edited by K. Kuwajima, M. Aria. Elsevier, Amesterdam, pp. 65-74.
Schneeman BO, Chang I, Smith L, Lyman LR (1977). Effect of an alpha-amylase inhibitor on the growth rate of weaning rats. Nutr. Reports Int., 15: 443
Scott ML, Neshim MC, Young RJ (1982). Nutrition of the chicken. Scott ML, Ithaca, NY. Pp. 394.
Sheppard A (2004). The structure and econoics of broiler production in England. Special studies in agriculture economics. University of Exeter Publication, p. 59.
Swick RA (2001). An update on soybean meal quality considerations. American Soybean Association.
Takahashi K, Mashiko T, Akiba Y (2000). Effects of dietary concentration of xylitol on growth in male broiler chicks during immunological stress. Poult. Sci. 79:743-747.
Vijiykumari K, Siddhuraja P, Pugalenthi M, Janardhanan K (1998). Effect of soaking and heat processing on the levels of antinutrient and digestible proteins in seeds of vigna aconitifolia and vigna senensis. Food Chem. 63(2):259-264.
Wedegaertner TC, Lalor WF (1997). Roasted cottonseed for dairy cows: Research review. In proc. Beltwide Cotton Fonf. P 1602. Natl. Cotton Counc. Am., Memphis, TN
Wiryawan KG, Dingle JG (1998). Recent research on improving the quality of grain legumes for chicken growth. Rev. Anim. Feed. Sci. Technol. 76:158-193.
Zhang YE, Parsons CM, Weingartner E, Wijeratne WB (1993). Effects of extrusion and expelling on the nutritional quality of conventional and kuntiz trypsin inhibitor-free soybeans. Poult. Sci. 72:2299-2308.