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## Full Length Research Paper

# Main and interaction effects of extrusion temperature and usage level of full fat soybean on performance and blood metabolites of broiler chickens

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The extruded full fat soybean (EFFSB) may be used in diet to satisfy the energy and protein requirements of fast growing broiler chickens. The main and interaction effects of three extrusion temperatures and two dietary levels of FFSB were studied on the performance, physiological enzymes and blood metabolites of broiler chickens from 0 to 42 days. The batches of full fat soybean (FFSB) were wet extruded at three temperatures (145, 155 and 165°C, respectively) for 15 s. 240 day-old Ross 308 male broiler chicks were divided and placed in 24 pens. Six starter, grower and finisher diets were provided in a 2 × 3 factorial design with two dietary levels of EFFSB (7.5 and 15%) and three extrusion temperatures (145, 155 and 165°C). The extrusion temperatures did not have significant effect on feed intake (FI), body weight gain (WG) and feed conversion ratio (FCR) of chickens during each and whole period, but the inclusion rate of EFFSB had significant ( $P < 0.05$ ) effect on FI and WG. The FI and WG of chickens fed diet contained 15% EFFSB was significantly ( $P < 0.05$ ) lower than those fed 7.5% EFFSB diet. The extrusion temperatures and inclusion rate did not significantly affect pancreas weight, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, indicating that the anti-nutrients residuals of FFSBs extruded at 145, 155 and 165°C did not influence liver system and pancreas size of chicks at 21 days of age. The interaction effects between dietary levels of EFFSB and extrusion temperatures on performance criteria, pancreas weight, CPK, LDH, AST and ALT enzymes of chickens were not significant ( $P > 0.05$ ) during each and whole period. It is concluded that blood metabolites and broiler performance were not affected ( $P > 0.05$ ) with extrusion temperature from 145 to 165°C, but increasing dietary levels of EFFSB from 7.5 to 15%, affected ( $P < 0.05$ ) negatively the growth rate of chicks.

**Key words:** Full fat soybean extrusion, temperature, broiler performance, blood metabolites.

## INTRODUCTION

The term full-fat soybean (FFSB) refers to soybean seeds that have been heat processed prior to oil extraction. Recently, interest has developed in the use of FFSB as a replacement for both soybean meal and fat in broiler chicken diets and using FFSB may eliminate the cost of oil extraction and allow the use of a homegrown protein supplement in the poultry diets (Arnold et al., 1971; Simovic et al., 1972; Leeson et al., 1987; Laudadio and

Tufarelli, 2011). Unprocessed FFSB contain anti-nutritional factors (ANFs) particularly trypsin inhibitor, lectins and saponins, which limit its use in poultry diets (Monari, 1996). Rackis (1974) reported that trypsin inhibitors may reduce the activity of trypsin and chymotrypsin leading to growth depression and pancreas enlargement in chicken (Liener, 1994).

However, thermal treatment of FFSB can inactivate the trypsin inhibitors and lectins (Sathe et al., 1984). Some researchers reported that FFSB protein causes changes in cholesterol metabolism, but some others showed that fibre and isoflavones of FFSB may change the cholesterol

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metabolism either directly or indirectly (Potter, 1995). Soy lectins in raw FFBS may damage liver system (Kayla, 2005). Increasing the amount of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) which are released from liver to blood stream of chicken is an indicator of liver damage (Chatila and West, 1996). One of the effective heat treatments is extrusion system including high temperature, high pressure and short period of time (Bjorck and Asp, 1983). Wet extrusion requires pre-conditioning as steam injection. Even after extruding FFBS in common temperatures, residual concentrations of some ANFs may be found in EFFSB and this may cause some negative effects on chicken performance when high levels of dietary EFFSB is fed.

The objectives of heating processes of FFBS are to maintain balance between degradation of anti-nutrients on the one hand and maintaining a good bioavailability of essential amino acids on the other hand (Kaankuka and Balogun, 1996). Therefore, the aim of this experiment was to study the effects of extrusion temperature, dietary inclusion rate of EFFSB and interaction effects between them on performance, physiological enzymes and blood metabolites of broiler chickens.

## MATERIALS AND METHODS

### Extrusion of full fat soybean

The batches of whole full fat soybean were wet extruded (EFFSB) at 145, 155 and 165°C for 15 s using an extruder system (Yemmak Co. Turkey). After extruding, two samples from each batch of EFFSB were analyzed for dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF), organic matter (OM) and ash according to AOAC (1990) and amino acids contents estimated with NIRS (Evonic, Corp. Germany).

### Management of birds

240 day-old Ross 308 male broiler chickens obtained from a local hatchery. The chicks were randomly distributed and assigned to six dietary treatments in a 2 × 3 factorial design including two dietary levels of EFFSB (7.5 and 15%) and three extrusion temperatures (145, 155 and 165°C) with four replicates and 10 chicks each. Dried wood shavings were used as litter at a depth of about 5 cm on floor pens. Each replicate group of birds was randomly placed in a pen (1.2 × 1.2 m) with one hanging feeder and one water cup. Birds had free access to feed and water at all times. The birds were exposed to 24 h lighting photoperiod throughout the experiment. Initial room temperature was 32°C and was gradually decreased according to the usual commercial practices. The birds were fed isocaloric and isonitrogenous starter, grower and finisher mash diets formulated according to AVIAGEN recommendations for Ross 308 broiler chickens Aviagen, (2007), using EFFSB chemical analysis determined in this study (Table 1).

### Performance criteria

The average daily feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) for each group of birds were calculated for each period. Daily mortality was weighed, recorded and used to correct the FCR.

### Weight of pancreas

One bird per replicate with average pen weight was selected, weighed, sacrificed, plucked and eviscerated on day 21 and 42. The pancreas was removed, weighed and expressed as a percentage of live weight.

### Blood metabolites and enzymes

At 21 days of age, one chicken from each replicate with average pen weight was selected. Blood samples was taken from wing vein, kept at the room temperature for 30 min and then centrifuged for 15 min at 3000 rpm to obtain serum and plasma, which was transferred to Eppendorf tube and kept at -20°C until analyzed by an auto-analyzer system (Bio Systems S. A. – Costa Brava 30, 08030 Barcelona, Spain) using commercially available kits (Bio Systems Co. Spain). The blood serum from each bird was used to determine AST, ALT, lactate dehydrogenase (LDH), creatine kinase (CK), low density lipoprotein (LDL), high density lipoprotein (HDL), cholesterol and triglyceride.

### Statistical analysis

The main and interactions effects between factors (2 levels of EFFSB × 3 extrusion temperatures) were analyzed by analysis of variance (ANOVA) in a 2×3 factorial design using the procedure described by the Statistical Analysis System (SAS) Institute (2008). The percentage values of pancreas weight were transformed to arcsine values and then subjected to analyses. Duncan means separation test (Duncan, 1955) was used to determine significant differences between treatment mean values ( $p < 0.05$ ). The relationship between extrusion temperatures and measured criteria was tested for linearity using SAS software (2008).

## RESULTS AND DISCUSSION

### Performance criteria

The effects of extrusion temperatures, dietary inclusion rate of EFFSB and their interactions on daily FI, WG and FCR are shown in Table 2. Extrusion temperatures did not affect FI, WG and FCR of chickens during starter, grower and finisher periods, as well as in the whole period. However, the dietary inclusion rate of EFFSB influenced significantly the FI during finisher and whole periods. Birds fed diets containing 15% EFFSB consumed less ( $p < 0.05$ ) feed than those fed 7.5% EFFSB diet (86.0 vs. 91.5 g/b per day). Leeson et al. (1987) reported a decrease in feed consumption of chickens fed diet containing high levels of heat treated FFBS. Also, Hamilton and Sandstedt (2000) found that feed intake of chickens was reduced with increasing levels of heat treated FFBS. Leeson et al. (1987) roasted FFBS at 118°C and showed that birds fed diets containing FFBS consumed less feed during 0 to 21 days of age, especially in response to high inclusion rates of FFBS. Growth rate of chickens fed 15% EFFSB diet was significantly ( $p < 0.05$ ) reduced when compared with those fed diet contained 7.5% EFFSB.

In a previous study, Papadopoulos and Vadoros

**Table 1.** Composition (%) of starter, grower and finisher diets.

Ingredient	EFFSB (%)					
	0 to 10 days		11 to 24 days		25 to 42 days	
	7.5	15	7.5	15	7.5	15
Corn	49.30	50.94	57.70	57.60	63.26	63.18
Soybean meal (dehulled)	36.00	29	29.40	23.41	24.41	18.16
EFFSB	7.50	15	7.5	15	7.5	15
Soybean oil	3.00	1	1.91	0.5	1.31	0.2
Sodium chloride	0.44	0.44	0.42	0.41	0.41	0.40
Limestone	1.36	1.36	1.05	1.02	1.05	1.01
Dicalcium phosphate	1.66	1.66	1.32	1.36	1.35	1.34
DL-methionine	0.14	0.14	0.10	0.10	0.11	0.11
L-Lysine HCL	0.10	0.10	0.10	0.10	0.10	0.10
Min + Vit premix <sup>1</sup>	0.50	0.50	0.50	0.50	0.50	0.50
<b>Calculated content</b>						
ME (kcal/g)	2950	2950	3000	3000	3050	3050
Crude protein (%)	22.68	22.68	20.69	20.69	19.06	19.06
Linoleic acid (%)	1.22	1.22	1.14	1.14	0.95	0.95
Crude fiber (%)	3.36	3.96	3.33	3.76	3.23	3.66
Calcium (%)	1.02	1.02	0.86	0.86	0.81	0.81
Available P (%)	0.49	0.49	0.43	0.43	0.40	0.40
Sodium (%)	0.19	0.19	0.18	0.18	0.17	0.17
Arginine (%)	1.28	1.28	1.09	1.09	0.97	0.97
Lysine (%)	1.24	1.24	1.05	1.05	0.92	0.92
Met + Cys (%)	0.46	0.46	0.40	0.40	0.36	0.36
Met + Cys (%)	0.92	0.92	0.80	0.80	0.72	0.72
Threonine (%)	0.81	0.81	0.70	0.70	0.62	0.62
Tryptophan (%)	0.20	0.20	0.17	0.17	0.15	0.15

<sup>1</sup>Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 9,790 IU; vitamin E, 121 IU; vitamin B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; copper sulfate, 100 µg; selenium (sodium selenate), 0.2 mg; iodine, 1 mg; manganese oxide, 60 mg.

(1988) using heat treated FFSB in broiler diets up to 15% reported that the body weight at 6 week of age was not adversely affected; but in our study, the FI and WG of birds fed 15% was reduced ( $p < 0.05$ ). The FCR of broiler chickens was not affected ( $p > 0.05$ ) by level of EFFSB or extrusion temperature. Waldroup et al. (1985) also used 10 and 20% of heat-treated FFSB in diet and observed that chicks fed 20% heat-treated FFSB had lower FI, higher WG and better FCR as compared to those fed 10% heat-treated FFSB. In addition, Wang et al. (2000) showed that birds fed diets containing 15% raw FFSB caused a significant decrease in WG when compared with control and extruded soybean diets. They revealed that the effect of feeding 15% extruded soybean on growth performance was similar or superior than those fed 15% soybean meal combined with soybean oil. The reduction of animal performance when raw soybeans were fed is normally attributed to the presence of anti-nutritional compounds (primarily the trypsin inhibitors) and to the highly folded structure of the soybean proteins,

which impede the proteolytic action of trypsin and chymotrypsin (Rackis et al., 1986; Monari, 1996). Proper processing of FFSB requires control of temperature and processing time (Wright, 1981) to inactivate FFSB anti-nutritional factors. On the other hand, excessive heat or heating time reduces the availability of amino acids due to the Maillard reaction (Del Valle, 1981) and tends to destroy certain amino acids (Chae et al., 1984) which may cause a poor growth.

Moreover, Ravindran and Blair (1992) reported that high temperature with mechanical pressing may damage FFSB protein, destroy amino acids and decrease their availability; therefore proper processing of FFSB requires control of temperature and processing time. Leeson and Atteh (1996) extruded FFSB at 140°C and observed a reduction in concentration of antitrypsin from 58.7 mg/g in raw beans to 8.4 mg/g for extruded heated beans. The acceptable level of antitrypsin factor is 4 mg/g; this level seems not to have any adverse effect on performance in broiler chickens. Perilla et al. (1997) revealed that trypsin

**Table 2.** Effect of extrusion temperature (ET) and dietary inclusion rate (IR) of EFFSB on feed intake (FI), body weight gain (WG) and feed conversion ratio (FCR) of broiler chickens from 0 - 42 days.

Main effect	Feed intake (g/b/d)				Weight gain (g/b/d)				Feed conversion ratio (g:g)				
	0 to 10 days	15 to 24 days	25 to 42 days	0 to 42 days	0 to 10 days	15 to 24 days	25 to 42 days	0 to 42 days	0 to 10 days	15 to 24 days	25 to 42 days	0 to 42 days	
ET (°C)	145	16.39	66.86	143.43	87.34	11.06	39.52	68.72	45.12	1.483	1.695	2.088	1.936
	155	16.54	72.00	148.66	91.27	10.58	39.79	65.68	43.63	1.565	1.705	2.280	2.096
	165	15.72	64.63	146.27	87.68	10.66	40.21	72.33	46.79	1.476	1.610	2.031	1.976
	SEM	0.333	2.338	2.691	1.519	0.284	1.149	1.986	1.026	0.040	0.033	0.072	0.051
	P-value	0.225	0.114	0.415	0.170	0.463	0.913	0.099	0.136	0.269	0.085	0.076	0.088
IR (%)	7.5	16.89	70.33	150.64 <sup>a</sup>	91.52 <sup>a</sup>	10.91	40.15	72.12 <sup>a</sup>	46.56 <sup>a</sup>	1.55	1.75	2.10	1.97
	15	16.54	65.33	141.60 <sup>b</sup>	86.01 <sup>b</sup>	10.63	39.53	65.70 <sup>b</sup>	43.80 <sup>b</sup>	1.46	1.65	2.16	1.96
	SEM	0.272	1.909	2.197	1.240	0.232	0.938	1.621	0.838	0.033	0.037	0.059	0.042
	P-value	0.204	0.089	0.013	0.008	0.423	0.647	0.016	0.037	0.073	0.093	0.503	0.883
ET × IR	SEM	0.472	3.306	3.805	2.148	0.402	1.625	2.808	1.452	0.057	0.047	0.102	0.072
	P-value	0.133	0.084	0.086	0.082	0.862	0.577	0.293	0.719	0.408	0.307	0.514	0.237

<sup>a,b</sup>Means in column with no common superscript differ significantly ( $P < 0.05$ ); Values were means for four replicates;  $N = 4$ .

inhibitor was reduced from 37.92 mg/g in raw FFSB to <0.1 mg/g in FFSBs when the extruding temperature was 140°C. They found also high correlation between trypsin inhibitor activity and weight gain ( $R^2 = 0.95$ ), and significantly lower ( $p < 0.05$ ) body weight gains were obtained when FFSB were extruded at 120 and 118°C compared with FFSB extruded at 122 and 126°C for 20 s. In our study, although the extrusion temperatures (145, 155 and 165°C) for 15 s, could not influence feed intake of chickens during whole period (0 to 42 day), but at inclusion level of 7.5%, EFFSB was observed to be linearly decreased significantly in feed intake of chickens with increase in temperature of extrusion during starter period (0 to 10 days of age). Extrusion temperatures (145, 155 and 165°C) did not affect weight gain of chickens during each and whole periods.

### Pancreas weight

The Kunitz and Bowman-Birk inhibitors are the two major types of trypsin inhibitors in FFSB that may increase enzyme secretion in young chicks, leading to increasing pancreas weight and size (Liener, 1994). The effects of extrusion temperature, dietary inclusion rate of EFFSB and their interaction on pancreas weight (as percentage of weight) of chickens are shown in Table 3. Results show no effects ( $p > 0.05$ ) of extrusion temperature, dietary inclusion rate of EFFSB or their interactions on pancreas weight of chickens. Furthermore, no linearity trend was observed between pancreas weight and increase in the extrusion temperature in 21-day chicks. Leeson et al. (1987) reported that the detrimental effects of feeding high levels of heat treated FFSB were less severe

as broiler chickens aged. Most enzymes are produced by pancreas for digestion of feed; therefore factors affecting pancreatic function may markedly influence the availability of nutrients in chickens. Feeding trials with rats (Friedman et al., 1991), chicks (Zhang et al., 1993), laying hens (Zhang et al., 1991), and pigs (Cook et al., 1988) have demonstrated that the inclusion of raw Kunitz Free soybeans in a diet resulted favorably in terms of growth traits compared with raw soybean. Perilla et al. (1997) also reported that relative pancreas weight decreased quadratically ( $p < 0.05$ ) as extrusion temperature increased and the highest relative pancreas weight was observed in chickens receiving raw FFSB. Moreover, Clarke and Wiseman (2007) observed a negative linear relationship between pancreas weight of chickens and extrusion temperatures from 90 to 160°C

**Table 3.** Effect of extrusion temperature (ET) and dietary inclusion rate (IR) of EFFSB on creatine phosphokinase (CPK), lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and pancreas weight of broiler chickens at 21 days of age.

Main effect	CPK (U/L)	LDH (U/L)	SGOT (AST) (IU/L)	SGPT (ALT) (IU/L)	Pancreas weight (as percentage of weight)	
ET (°C)	145	8349.16	3985.66	250.33	11.16	0.323
	155	8861.83	4550.00	263.00	10.46	0.339
	165	8009.16	4257.66	252.83	11.33	0.322
	SEM	380.716	511.798	16.719	0.483	0.015
	P-value	0.317	0.743	0.853	0.430	0.705
IR (%)	7.5	8887.22	4149.77	258.88	11.08	0.310
	15	8926.55	4377.77	251.88	10.88	0.346
	SEM	310.855	417.887	13.656	0.390	0.012
	P-value	0.066	0.706	0.723	0.726	0.074
ET × IR	SEM	538.415	723.793	23.645	0.683	0.022
	P-value	0.409	0.927	0.700	0.386	0.266

### Physiological enzymes

The effect of extrusion temperature and dietary inclusion rate of EFFSB and their interactions on the activity of ALT, AST, creatine phosphokinase (CPK) and LDH in chicks at 21 days of age are reported in Table 3. The liver ALT and AST in blood serum of chickens were not influenced by extrusion temperature or inclusion rate of EFFSB. Moreover, there were no effect ( $P > 0.05$ ) of extrusion temperature and dietary inclusion rate of EFFSB or their interaction on CPK and LDH of 21 d chicks. Increasing amount of AST or ALT released from liver to blood stream of chicken is an indicator of liver damage (Chatila and West, 1996). These enzymes were reported to increase with increasing crude oil concentrations in diet and have been related to the extent of hepatic, cardiac and kidney damage in treated subjects, (Chambers et al., 1978). The liver contains many blood vessels that rupture easily during egg laying, resulting in massive bleeding and death.

Furthermore, Kayla (2005) reported that the liver may be influenced by the lectins of soybean. Blood serum LDH and CPK concentrations are elevated in association with the failure of cardiovascular system, and these changes are used as indicators of diagnosis of circulatory disturbance (West et al., 1966). In broiler chickens, the CPK is released into the blood stream in response to various pathogens and exposure to environmental stressors (Bogin et al., 1997). Also, infections may cause an increase in LDH (Smith, 1987). The LDH level is directly correlated to growth rate (Khajali and Qujeq, 2005). When large amounts of fat are deposited in the hen's liver and abdomen, the liver becomes soft and easily damaged and is more prone to bleeding. In our study, all diets had similar fat content during whole

periods and liver ALT and AST in blood serum of chickens were not significantly influenced by extrusion temperature or inclusion rate of EFFSB, indicating that the residual lectins up to 15% FFBSB extruded at 145, 155 and 165°C did not damage the liver system.

### Blood serum triglyceride and lipoproteins

The effects of extrusion temperatures and inclusion rate of EFFSB on blood serum lipoproteins and triglyceride of chickens are shown in Table 4. The concentration of triglyceride, cholesterol, HDL and LDL in blood serum of broiler chickens can be controlled by feed manipulation. In a study by Hermier and Dillon (1992), it was reported that serum lipoprotein concentrations could be changed by dietary fat in broilers. Based on the fact that the serum HDL carries about 75% of total cholesterol in chicks (Peebles et al., 1997), it is more likely that this lipoprotein may be more influence by the type of dietary fat. The very low density lipoprotein (VLDL) concentrations are relatively good indicators of fat deposition in the bird (Griffin and Whitehead, 1982). Diets containing high level of animal protein have been linked to increased cholesterol levels. Conversely, plant-derived protein (especially FFBSB) has been shown to decrease cholesterol. Some researchers reported that FFBSB was effective in lowering the levels of serum triglycerides and cholesterol in humans and animals (Anderson et al., 1995). However, the mechanism of FFBSB on blood cholesterol is unknown, although several theories have been proposed. One hypothesis suggests that the amino acid composition of FFBSB protein causes some changes in cholesterol metabolism. In other studies, some authors proposed that non-protein components (such as fiber and

**Table 4.** Effect of extrusion temperature (ET) and dietary inclusion rate (IR) of EFFSB on triglyceride (TG), cholesterol (CHOL), high density lipoprotein (HDL) and low density lipoprotein (LDL) of blood serum of 21- day old broiler chickens.

Main effect		TG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
ET (°C)	145	91.00	128.83	94.00	26.83
	155	91.83	128.66	104.33	24.33
	165	84.83	121.50	95.50	24.00
	SEM	8.151	4.535	4.177	3.187
	P-value	0.806	0.451	0.209	0.793
IR (%)	7.5	98.22	126.55	95.44	28.55
	15	80.22	126.11	100.44	21.55
	SEM	6.655	3.703	3.410	2.602
	P-value	0.080	0.934	0.320	0.081
ET x IR	SEM	11.526	6.413	5.906	4.507
	P-value	0.609	0.414	0.790	0.512

isoflavones) associated with FFSB protein affect cholesterol metabolism either directly or indirectly (Potter, 1995).

Isoflavones have been implicated as the components of FFSB that improve lipid profiles. On the other hand, the dietary fiber fraction in FFSB has been reported to be an important component that could reduce serum cholesterol levels (Uberoi et al., 1992). The soluble and insoluble non-starch polysaccharides (NSP) of legume seeds are known to be an effective cholesterol-reducing agent (Hughes, 1991). The carbohydrates in soybean consist of approximately 10% free sugars (5% sucrose, 4% stachyose and 1% raffinose) and about 20 to 30% NSP (Choct, 1997) that are soluble and can increase digesta viscosity, reduce digestibility of lipids (Choct et al., 2010) and cause to reduce serum cholesterol concentration (Uberoi et al., 1992). The most popular theory suggests that FFSB protein reduces cholesterol metabolism in the liver by increasing the removal of LDL 'bad' cholesterol (Sirtori et al., 1995). In our study, the percent of crude fiber and crude fat of all diets were similar, therefore the effects of extrusion temperature and inclusion rate of EFFSB could not significantly ( $p>0.05$ ) influence the blood serum lipoproteins and triglycerides.

## Conclusion

In general, the effect of extrusion temperature (145, 155 and 165°C) of FFSB did not influence performance of chickens during the whole period. Inclusion dietary rate of EFFSB from 7.5 to 15% decreased FI and WG of broiler chickens. The extrusion temperatures and inclusion rate of up to 15% EFFSB did not affect pancreas weight and blood parameters, revealing that the anti-nutrients residuals of FFSB extruded at 145, 155 or 165°C were lower than the threshold to influence liver system and pancreas size in chicks at 21 days of age.

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