Asian Journal of

Poultry Science

ISSN 1819-3609

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2018.14.24

Review Article

Anti-nutrients Reduce Poultry Productivity: Influence of Trypsin Inhibitors on Pancreas

Mammo M. Erdaw and Wude Ts. Beyene

Ethiopian Institute of Agricultural Research, Debre-Zeit Agricultural Research Center, Ethiopia

Abstract

Foods of animal-products for the consumers have recently and mainly been from the non-ruminant animals (poultry). However, presence of anti-nutrients, in feeds is one of the constraints that impairing them from further productiveness. The study aimed to disclose both of the adverse effects of anti-nutrients and suggested solutions. Related findings of this topic were scientifically reviewed and summarized to being publicized. This review revealed that there are a range of anti-nutrients that are synthesized by plants as part of protections or as means to survive. Anti-nutrients that interfere with normal animals' physiological activities could have anti-nutritional effects. Trypsin Inhibitor (TI) is one of the anti-nutrients and is almost nil in cereal grains; whereas highly concentrated in legumes, mainly in soybean grains (2-6 mg g^{-1}). Kunitz-trypsin-inhibitors (KTI) and Bowman-Birk Inhibitors (BBI) are abundant of TI, containing 181 and 71 AA, respectively. The KTI and BBI contained 1.4 and 1.6 g kg^{-1} of seed, respectively. Although, TI in the diets of chicks, rats and mice caused for pancreatic hypertrophy and increased secretions, others including pigs, dogs or pre-ruminant calves were not experiencing these symptoms. A human endogenous-trypsin enzyme has also more resistance to TI as compared to other mammals. Although most anti-nutrients are suggested to be inactivated by heat treatments, over or under-heating affects those other important nutrients. The BBI, as part of TI always exhibits considerable resistance to heating. Other anti-nutrients, for example phytate is totally heat-stable. As TI contributes a major-proportion of sulfur-containing amino-acids (AA), soybean breeding for a low TI has limited application. When commercial soybean meal was replaced by raw soybeans (0-90 g kg^{-1} diet), the weights of pancreas and duodenum were proportionally increased by 67 and 21%, respectively. The TI in diets of poultry affects the pancreas, but TI could be reduced by either optimum heating or enzyme supplementations.

Key words: Anti-nutritional factors, trypsin-enzyme, pancreas, productivity, non-ruminant animals, trypsin inhibitors, sulfur-containing AA

Citation: Mammo M. Erdaw and Wude Ts. Beyene, 2018. Anti-nutrients reduce poultry productivity: Influence of trypsin inhibitors on pancreas. Asian J. Poult. Sci., 12: 14-24.

Corresponding Author: Mammo M. Erdaw, Ethiopian Institute of Agricultural Research, Debre-Zeit Agricultural Research Center, Ethiopia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Globally the non-ruminant animals have currently been taking the major parts in supplying and satisfying to the skyrocketing demands of protein source foods of animal origins. These are mainly originated from the farm animals. However, lack of proper utilization of the available feed resources, in developing countries is one of the constraints that are negatively affecting these animals from their further enhanced productivities. Due to many reasons, protein source feeds for these animals have recently and mainly been derived from the plants. Of which, legume grains, in particular are good sources of plant protein feeds. However, presence of anti-nutritional factors (ANF) or anti-nutrients, in such feed ingredients is one of the factors negatively affecting the feed quality.

These ANF cause for adverse physiological and functional effects of the animals when they consume the feeds containing the ANF that are more than the threshold levels[1]. As compared to other internal organs, many types of digestive enzymes are synthesized by the exocrine pancreas, stored in the zymogen granules and released into the duodenum[2]. However, ANF elicit their negative effects via different mechanisms, including binding to digestive enzymes and nutrients or increasing the gut viscosity[3]. In addition to the heat treatments, optional solutions to these problems may lie with the use of microbial feed enzymes[3-5]. This review paper attempted to review the findings on the topic and summarize them and then publicize to be used by the users.

DESCRIPTION OF ANTINUTRITIONAL FACTORS

Depending upon the structures of the individuals, a range of secondary metabolites (ANF) are always synthesized by plants as part of protection against the attacks by the other organisms or as a means to survive in adverse growing conditions. These are toxic compounds, with varying effects[6] on animals when they consumed. These ANF are not only found in grains but also present in different edible leafy vegetables plants[7] too.

Anti-nutritional factors can be classified mainly on three major categories, namely on their chemical descriptions, on their biological effects and on their ability to withstand heat treatments.

In the first place, when the ANFs are classified based on their chemical structures, they are grouped as: (a) Proteins (protease inhibitors and haemagglutinins (lectins), (b) Glycosides (glucosinolates, cyanogens, saponins and estrogenic factors), (c) Phenol (gossypol, tannins) and (d) Miscellaneous[8]. Secondly, when Francis *et al.*[9] classified the ANF based on their biological effects, the ANF were grouped into three categories, such as (1) Protein utilization and digestion (i.e., protease inhibitors, tannins and haemagglutinins (lectins), (2) Mineral utilization (i.e., phytic acid) and (3) Anti-vitamins and miscellaneous (i.e., mimosine, cyanogens and estrogenic factors). Lastly, when the ANF are being described based on their heat-resistance[9], they are grouped as heat-labile factors (i.e., protease inhibitors, haemagglutinins and anti-vitamins) and as heat-stable factors (e.i., saponins, non-starch polysaccharides, anti-genic proteins, estrogens and some phenolic).

A wide variety of ANF, such as oligosaccharides, proteinaceous compounds (i.e., Trypsin Inhibitors (TI)), lectins, amylase inhibitors and phytic-acids are mainly found in legume grains[10], such as soybean, peas, faba-bean, lupins, common vetch, grass pea and kidney bean, which are commonly used as feed ingredients for the non-ruminant animals. Protease inhibitors (i.e., TI), lectins and phytate are the best characterised ANF in soybeans[11]. In addition to negatively affecting the physiological and functionalities of internal organs, these ANF influence the bioavailability and absorption of nutrients to the animals when they are used as feeds. Guillamon *et al.*[12] reported that the contents of TI ranged from negligible in cereals or *Lupinus* spp. to higher amounts in soybean cultivars (43-84 TIU mg^{-1}) followed by common beans (21-25 TIU mg^{-1}).

Heat treatment is generally considered as an effective approach to inactivate ANF but some of the ANF, e.g., phytate, oligosaccharides and antigenic proteins, cannot be reduced or decreased by heating[3]. Although, Campbell and Schone[13] suggested that TI and lectins in soybeans could be inactivated by heating, Clemente *et al.*[14] reported that Bowman-Birk Inhibitors (BBI), which are a group of TI, exhibit considerable resistance to the heat treatment.

Lectins are widely distributed in legumes and in some oilseeds[23] but it can be effectively treated by heating, mainly in an aqueous medium (i.e., 100°C for 10-20 min). Limited numbers of ANF are shown in Table 1.

PHYSIOLOGICAL AND FUNCTIONAL EFFECTS OF ANTINUTRITIONAL FACTORS

If animals or humans consume the ANF in plants, these compounds may cause adverse physiological and functional effects. These ANF negatively affect the nutritive values of the leguminous grains through direct and indirect reactions. They inhibit proteins and carbohydrate digestibilities and then even they induce pathological changes in the intestine and liver tissues. Thus, ANF affects the metabolism and inhibits a number of enzymes and then bind nutrients, making them

Table 1: Selected anti-nutrients in both raw full-fat soybeans and commercial soybean meals

Raw, full-fat soybeans				Commercial soybean meals			
TI ($mg\ g^{-1}$)	UA (ΔpH)	Lectins ($mg\ g^{-1}$)	Phytate ($mg\ g^{-1}$)	TI ($mg\ g^{-1}$)	UA (Δ pH)	Phytate ($mg\ g^{-1}$)	References
40.486	>0.20	3.7	-	-	0.05-0.20	-	Gu *et al.*[15]
30-102.5	-	-	5-25	-	-	14.5	Sharma *et al.*[16]
45-60	-	20-200 (ppm)	6	1.77	.02	-	(Van Eys *et al.*[17]
26.48	-	-	-	1.8-2.9	-	-	Serrano *et al.*[18]
48.33 (TIU mg^{-1})	-	-	11.2	-	0.2	-	Crowell[19]
-	-	-	-	2.5-1.9 (TIU mg^{-1})	~0.01	-	Ravindran *et al.*[20]
23.9	-	7.3	-	-		-	Crowell[19]
50,800 (TIU g^{-1})	1.99	-	-	3,000 (TIU g^{-1})	0.08	-	Ruiz *et al.*[21]
41.5-85	-	-	2.3-5.6	1.50-3.45	-	-	Sharma *et al.*[22]

TI: Trypsin inhibitors, UA: Urease activity, phytate, TIU: Trypsin inhibitor units

unavailable[24]. Various scholars[25-28] have reported that feeding raw soybean with high levels of TI and lectins negatively affects the pancreatic functions, the growth of birds and the feed efficiency. Mogridge *et al.*[29] reported also that the consumption of raw beans increased the size of the pancreas and duodenum (0.80 vs. 0.37% and 1.35 vs. 1.06% of live weight, respectively) and reduced feed consumption and growth of the chicks (66 vs. 97 g/14 day).

Similarly, ASA[30] reported that diets based on raw beans reduced the feed consumption and live weight. Availability of ANF within plant feedstuffs is the main factors that limit their use by the animals[8,31]. Emiola *et al.*[32] studied the effect of feeding birds with raw and dehulled meals and then found an increased weight of pancreas, severe congestion of kidney, reduced weight of liver with its marked coagulative necrosis. Dietary ANFs have also adversely affected the digestibility of protein, bioavailability of Amino Acids (AA) and protein quality[33]. The TI in the diet of chicks, rats and mice caused pancreatic hypertrophy and increased pancreatic secretions. However, pigs, dogs or pre-ruminant calves were not experiencing these symptoms when consuming TI in their diets. One of the effects of the inactivation of digestive enzymes in the intestine is the stimulation of trypsin and chymotrypsin secretion from the pancreas, which can create an increased demand for the sulfur AA methionine and cysteine[34].

The exocrine function of the pancreas is to produce and secrete several digestive enzymes, including trypsinogen, amylase, lipase and procarboxypeptidase, among others[35]. These enzymes are released into the ascending loop of the duodenum, certainly for digesting the nutrients[36]. As Shi *et al.*[37] stated the activities of pancreatic trypsin and chymotrypsin increased sharply from hatching to day 14 and then decreased gradually until day 21. The recent findings also confirmed this fact that the adverse effects of protease inhibitors on internal organs, particularly pancreas is aggravated at early ages[38-39]. Hence, age must be considered as a factor while planning to replace the commercial SBM by raw soybean in the diets of on-ruminant animals, including chickens. Although, anti-nutrients/ANF, in plant are responsible for deleterious effects that related to the absorption of nutrients and micronutrients, some anti-nutrients, at some extent with low concentrations may exert beneficial health effects by reducing the blood glucose and insulin responses[40].

Trypsin inhibitors: Trypsin Inhibitors (TI) are a type of serine protease inhibitor that reduces the biological activities of endogenous trypsin. Protease inhibitors are widely occurred across the plant kingdom and are protein-based molecules[23]. The concentration of TI is almost nil in cereal grains, otherwise highly concentrated in legumes, for example; TI are ranging between 2-6 mg g^{-1} in soybean[9,41]. The ANFs are found not only in soybeans but also in other legumes, including kidney beans[42].

From various categories of plant protease inhibitors, the main inhibitors in legume seeds and cereals are the trypsin and chymotrypsin inhibitors[43]. In soybeans, there are two main groups of TI, such as the heat-labile, which is called Kunitz trypsin inhibitors (KTI). This type of inhibitors is blocking mainly the trypsin and the second one is BBI, which inhibits both trypsin and chymotrypsin[9,23]. Trypsin is an endogenous enzyme involved in the breaking down of many different proteins, including as part of digestion in humans and other animals. As a result, protease inhibitors that interfere with activities of endogenous trypsin can have anti-nutritional effects.

The most abundant TIs are the KTI and BBI, containing 181 and 71 AA, respectively. However, concertation of these two in a typical seed grain is a reciprocal to their number of AA contained. The concentration of KTI and BBI are 1.4 and 1.6 g kg^{-1} of the total seed contents, respectively[44]. The same authors reported that soybeans also contain between 6.8 and 17.5 g of phytic acids/kg. This is a ring form of phosphorus (P), which chelates with proteins and minerals to form a phytate not be readily digested within the gut of non-ruminants.

Campbell and Schone[13] suggested that TI and lectins in soybeans could be inactivated by heating. Furthermore, moist heating (autoclaving at 121°C for 15-30 min) or aqueous heat treatment (100°C for 10 min) is preferably be recommended by other scholars[46,47]. However, Clemente *et al*.[14] reported that BBI, which is a group of TI, exhibits a considerable resistance to heat treatment. On the other hand, researches into breeding for low TI cultivars currently has limited application as trypsin inhibitors contribute a major proportion of sulfur containing AA including the methionine and cysteine content of soybeans[44].

Optionally, the scholars, for example Pettersson and Pontoppidan[11] have differently suggested that by supplementing the diets, containing ANF with selected microbial feed enzymes, including protease and phytase could reduce the impacts of anti-nutritional proteins on the non-ruminant animals and thereby improve the digestibility of proteins.

The loss of endogenous protein has been found to be increased due to the activity of TI in the body consequently affecting the nitrogen balance[44]. Herkelman *et al*.[48] also noted that the presence of TI in diets can cause digestive diseases in non-ruminant animals.

A high level of TI in diets, which are mostly from the soybean origins have also been found to correlate with a rapid feed passage, which leads to a greater nitrogen excretion and hence poor litter quality[48,49]. Extrusion of faba bean reduced the *E. coli* count in the small intestine and tended to decrease damage to intestinal villi[50]. Susmel *et al*.[51] tested an *in vitro* degradation of proteinase inhibitors contained in raw soya bean, with rumen fluid and found that it was degraded at a much slower rate which suggested that the presence of the KTI leads to a higher residual TIA after rumen degradation and slightly lower microbial gas production.

EFFECTS OF TRYPSIN INHIBITORS ON PANCREASES

Commercial soybean meal (SBM) is considered to be the best protein source feeds for the non-ruminant animals[52],. But, the nutritive value of raw full-fat soybean (RFSB) or that of poorly processed soybean is poor due to the presence of the ANF, especially the TI that adversely affecting the pancreas[26,53]. When the TI is there, the pancreas often tries to compensate the problems by increasing its size to synthesize and then to secret such sufficient digestive enzymes[42]. The linear increase in pancreas size with increasing soybean trypsin inhibitors intake suggested a dose dependent response of broiler chickens[54, 55]. Additionally, Rocha *et al*.[56] reported that the pancreas of the broilers fed the diets containing RFSB was significantly heavier and the intestinal integrity, as well as structure was also impaired.

The exocrine pancreas is the organ with the highest level of protein synthesis in animals. Each day the pancreas produces liters of fluid filled with enzymes that are capable of breaking down nearly all organic substances[54]. The TI negatively affects the functions of the pancreas, in terms of synthesis and secretion of digestive enzymes, including proteases. As an optional solution, some geneticists and crop breeding companies have developed seeds that have very low anti-nutritional factors.

However, the TI are very rich in sulfur containing AA as they may account for about 40% of the total cysteine content of some legume protein[46], which could show that breeding may not be strategic solution to solve the negative effects of ANF, particularly the TI in soybeans.

However, Pettersson and Pontoppidan[11] reported that negative impact of TI in non-ruminant animal diets could be reduced by supplementing the diets with microbial protease. The adverse effects of raw soybeans was discovered and reported by Osborne and Mendel[58], that it could not support the growth in rats unless the seeds are cooked.

Feeding raw soybeans resulted in growth inhibition and enlarged the pancreases. Following such a discovery, Chernick *et al*.[59] found the reason why the chicks' pancreases were enlarged in sizes was due to the presence of TI in raw soybeans. This led the pancreas to being enhanced producing more enzymes, pancreatic hypertrophy and hyperplasia as a mechanism withstand the problem. When an endogenous trypsin is inhibited by the TI, cholecystokinin production is also automatically enhanced resulting in an increased production of the pancreatic digestive enzymes.

As reviewed and reported by Liener and Kakade[60], the consequences of TI on pancreases have been confirmed not only in chickens but also in rats, mice and young pigs. Grant[61] added that dietary soybean or the TI do alter pancreatic secretion, which lead to pancreatic hypertrophy and hyperplasia occur, not only in chicks but also in the young of a number of species, proved that they are given the raw soybeans.

Furthermore, Batterham *et al*.[62] reported that the growing pigs can tolerate dietary levels of at least 4.7 and 4.5 mg g^{-1} of trypsin and chymotrypsin inhibitors, respectively; otherwise the weights of the liver and pancreas were significantly affected. However, Struthers *et al*.[63] reported that the TI neither from raw soy flour nor from any other soy products produced pancreatic enlargement in pigs or monkeys. Flavin[64] suggested also that human trypsin is more resistant to inhibition than is the trypsin of other mammalian species. The effect of TI, from soybean does not appear to be a potential hazard on human trypsin.

Pancreatic hypertrophy, increased weight of small intestines and increment of the thicknesses of both villous and crypt of small intestine were found from the mice fed diet containing raw soya flour[65]. Hence, Baba *et al.*[66] concluded that major resection of the pancreas leads to disorders of the endocrine and exocrine pancreas. An investigation was conducted to identify whether DNA concentration was proportionally increased in samples of pancreatic hypertrophy due to the TI from soybean. Then, the results showed that the concentration of DNA or the ratio of tissue protein to DNA was not affected[28]. Therefore, the increase in weight of the pancreas is due to hypertrophy than hyperplasia[29,45,67]. That means pancreatic hypertrophy may be due to cell sizes increments than increases of cell numbers.

When the supplementation of the raw soybean increased in diets, the weight of pancreas and other internal organs, including the duodenum, gizzard, proventriculus and small intestines were increased (Table 2-6). Although, microbial protease supplementation did not significantly reduce the weight, their effects were shown with improved BWG of the chickens across the trials[45]. When commercial SBM was increasingly (0-90 g kg^{-1} diet) replaced by raw soybean, the weight of pancreas and duodenum were increased by 67 and 21%, respectively[39] at day 14. However, weight of pancreas was significantly reduced at day 24 when microbial protease was increased from 0-0.4 g kg^{-1} diet, containing 75 of raw soybean per kg of diet[57]. Moreover, weight of pancreas was significantly reduced at day 24 and tended at day 10 when increasing supplementation of microbial phytase from 0.1-0.3 g kg^{-1} diet (Table 6).

THE MECHANISMS OF ANTINUTRITIONAL FACTORS IN AFFECTING PHYSIOLOGICAL FUNCTIONS

Anti-nutritional factors elicit their negative effects on animals via different mechanisms, including binding to digestive enzymes and nutrients or increasing gut viscosity[3]. Phytate, for example impedes nutrient digestion through electrostatic mechanisms, which reduces the solubility of

Table 2: Effects of varyingly supplementing protease and raw full-fat soybean on the weights of visceral organs (g/100 g of body weight) of broilers at day 10

Main effects (g kg^{-1} diet)								
RFSB	Protease	S. intestine	Pancreas	Liver	G+P	Heart	Bursa	Spleen
0	0.1	7.8^{b}	0.543^{c}	4.2	3.8	0.92	0.15	0.08
0	0.1	7.8^{b}	0.543^{c}	4.2	3.8	0.92	0.15	0.08
30		8.2^{ab}	0.613^{b}	4.1	3.8	0.96	0.18	0.08
60		8.6^{a}	0.710^{a}	4.0	3.8	0.87	0.18	0.08
	0.1	8.6^{a}	0.616	4.3	3.9	0.92	0.17	0.09
	0.2	8.2^{ab}	0.612	3.9	3.7	0.91	0.16	0.07
	0.3	7.9^{b}	0.639	4.0	3.8	0.93	0.17	0.08
Pooled SEM		0.02	0.02	0.26	0.28	0.02	0.01	0.00
Sources of variation								
RFSB		*	***	NS	NS	NS	NS	NS
Protease		**	NS	NS	NS	NS	NS	NS
RFSB x protease		NS	NS	NS	NS	NS	p = 0.05	NS

S. intestine: Small intestine, G+P: Gizzard plus proventriculus, SEM: Standard error of mean, RFSB: Raw full-fat soybean, NS: Non-significant, *p<0.05, **p<0.01, ***p<0.001, Source: Adopted from Erdaw *et al.*[38]

Table 3: Effects of varying both pelleting method and levels of raw full-fat soybean on the weight gains of the visceral organs (g/100 g of body weight) at day 14

Main effects									
RFSB (g kg^{-1} diet)	Pelleting method	G+P	Pancreas	Duodenum	J+I	Heart	Liver	Bursa	Spleen
0		3.1^{b}	0.39^{c}	1.35^{b}	5.6	0.81	3.3	0.24	0.08
30		3.2^{b}	0.45^{c}	1.43^{b}	5.9	0.86	3.4	0.21	0.08
60		3.3^{ab}	0.54^{b}	1.54^{b}	6.1	0.86	3.4	0.20	0.08
90		3.6^{a}	0.65^{a}	1.63^{a}	6.2	0.89	3.4	0.25	0.09
	Steam-pelleted	3.5^{a}	0.53	1.52	6.3^{a}	0.85	3.4	0.24	0.08
	Cold-pelleted	3.1^{b}	0.49	1.45	5.6^{b}	0.85	3.4	0.22	0.09
Pooled SEM		0.05	0.02	0.03	0.09	0.02	0.04	0.01	0.00
Sources of variation									
RFSB		*	***	**	NS	NS	NS	NS	NS
Pelleting method		**	NS	NS	***	NS	NS	NS	NS
RFSB x pelleting		NS	NS	NS	NS	NS	NS	p=0.05	NS

G+P: Gizzard plus proventriculus, J+I: Jejunum plus ileum, SEM: Standard error of mean, RFSB: Raw full-fat soybean, NS: Non-significant, *p<0.05, **p<0.01, ***p<0.001, Source: Adopted from Erdaw *et al.*[39]

Table 4: Effects of varying the supplementation of both protease and raw-full-fat soybean on the weights of internal organs (g/ 100 g body weight) at day 24

Main effects								
RFSB (g kg^{-1} diet	Protease (g kg^{-1})	G+P	Pancreas	SI	Heart	Liver	Bursa	Spleen
0		2.77^{c}	0.21^{b}	4.03^{b}	0.56^{c}	2.58	0.20	0.08^{b}
45		3.65^{a}	0.35^{a}	5.57^{a}	0.71^{a}	2.91	0.24	0.11^{a}
75		3.36^{b}	0.36^{a}	4.84^{a}	0.65^{b}	2.73	0.19	0.08^{b}
	0	3.29	0.31	4.81	0.64	2.7	0.19^{b}	0.09
	0.2	3.23	0.30	4.71	0.64	2.8	0.23^{a}	0.09
SEM		0.10	0.02	0.19	0.02	0.09	0.01	0.01
Sources of variation								
RFSB		***	***	***	***	NS	NS	*
Protease		NS	NS	NS	NS	NS	*	NS
RFSB x protease		NS	NS	NS	NS	NS	NS	NS

G+P: Gizzard plus proventriculus, SI: Small intestine, SEM: Standard error of mean, RFSB: Raw full-fat soybean, NS: Non-significant, *p<0.05, ** p<0.01, ***p<0.001, Source: Adopted from Erdaw *et al.*[45]

Table 5: Effects of varyingly supplementing the raw full-fat soybean and protease on relative pancreas weight (g/100 g of the body weight) in relation to the body weight of broilers[45]

Main effects (g kg^{-1} diet)		Live body weight (days)			Weight of pancreas (days)		
RFSB	Protease	10	24	35	10	24	35
0		282^{a}	1,463	2,540	0.54^{c}	0.23^{c}	0.15^{b}
30		279^{a}	1,420	2,432	0.61^{b}	0.31^{b}	0.20^{a}
60		265^{b}	1,419	2,464	0.71^{a}	0.34^{a}	0.21^{a}
	0.1	270^{b}	1,424^{ab}	2,422	0.62	0.29	0.19
	0.2	275^{ab}	1,415^{b}	2,461	0.61	0.29	0.19
	0.3	282^{a}	1,463^{a}	2,549	0.64	0.29	0.18
Pooled SEM		1.64	8.63	21.4	0.03	0.02	0.01
Sources of variation							
RFSB		0.001	0.123	0.10	0.001	0.001	0.01
Protease		0.005	0.03	0.06	0.102	0.612	0.423
RFSB x protease		0.511	0.432	0.712	0.231	0.641	0.451

RFSB: Raw full-fat soybean, SEM: Standard error of mean

Table 6: Effects of the extra-dosing of protease and phytase on the weight of the pancreas broilers (g/100 g of the body weight) at d 10, 24 or 35 when 25% of SBM was replaced by raw soybean[57]

Main effects (g kg^{-1})		Pancreas weight (days)		
Protease	Phytase	10	24	35
0.0		0.72	0.37^{a}	0.24
0.2		0.67	0.36^{a}	0.24
0.4		0.71	0.25^{b}	0.23
	0.1	0.73	0.33^{a}	0.23
	0.2	0.69	0.34^{a}	0.24
	0.3	0.67	0.31^{b}	0.24
Pooled SEM		0.01	0.01	0.01
Sources of variation				
Protease		NS	***	NS
Phytase		0.06	*	NS
Protease×phytase		NS	NS	NS

NS: Non-significant, *p<0.05, **p<0.01, ***p<0.001

proteins; consequently, increasing the loss of endogenous nutrients[68]. The response of chickens and rats to the TI requires the mediation of the gastrointestinal tract and is independent of vagal intervention to the pancreas[69]. Pusztai[70] reported that since some lectins are heat stable and can survive going through the gut, their interaction with the gut surface epithelium can damage the gut at high dietary intakes and this may lead to digestive disorders/diseases in some instances. Many fish species have been affected by ANF, particularly the IT, which led to pancreas hypertrophy[41]. After a certain period however a compensation process stimulates trypsin secretion and it seems that with trypsin inhibitors levels below 5 mg g^{-1}, most cultured fish are also be able to compensate[9].

Because of approximately 65-80% of the total phosphorus in the soybean seed is bound to phytic acid or phytate, most of the minerals, particularly phosphorus (P) is biologically unavailable because poultry do not possess enough endogenous phytase to digest the complex[71]. proportional increment of pancreas weight, against to that of increasing levels of TI in diets might be an indicator of the physiological mechanism to coping the negative impacts of ANF on birds by enlarging the surface area of pancreas and thereby to produce more of indigenous enzymes, particularly protease[57,45].

Implication of antinutritional factors on the productivity of animals: Protease (trypsin) inhibitors can interfere with the biological activity of endogenous protease and thereby reduce the digestion of nutrients, mainly proteins[72]. The loss of endogenous protein has been found to be increased due to the activity of TI in the body, consequently affecting the nitrogen balance[73]. Herkelman *et al.*[48] noted that the presence of TI in diets can cause a digestive disease in non-ruminant animals. A high level of TI in diets, mostly of the soya origins, have also been found to correlate with a rapid feed passage, which leads to greater nitrogen excretion and resulted in poor litter quality[50].

Phytate (phytic acid) is another ANF that is present at high level in soybeans. Phytate impedes nutrient digestions by forming protein-phytate or protein-phytate complexes and is resistant to normal digestions[74]. Phytic acids have a negative impact on amino acid digestibility and have recently been shown to increase the endogenous nutrient losses in pigs and poultry. Anti-nutritional factors cause depression in growth; performance and can negatively affects the health of the animals. Although tolerance depends on the age of the birds[30], excessive levels of the ANF reduce the nutritional quality of the feeds. However, some of the ANF can be denatured by heating[26].

MICROBIAL FEED ENZYMES IN REDUCING THE ADVERSE EFFECTS OF ANTINUTRITIONAL FACTORS

When animals lack specific enzymes to break down certain feed components, ANF interfere with the normal digestion process when they are present in the diet[75]. Birds fed on diets containing raw soybeans do not thrive. Their feed consumption rate is reduced by as much as 14%, their live weight gain drops by up to 15% and the feed conversion rate decreases by approximately 53%[30].

The mechanism and effectiveness of the microbial protease and phytase on raw soya proteins are not yet clear but their relative effectiveness, in reducing the adverse effects of TI and phytates in RFSB. This is a confirmation of results previously obtained in *in vitro* and *in vivo* studies[28,57,67]. Supplementing the diets with microbial phytase however breaks the bonds of phytic acids to release the nutrients, such as minerals. Consequently, the physical parameters and mineral composition of tibia bones of broilers were improved when microbial phytase is added to broilers' diets[76].

Proteases are protein-digesting microbial enzymes that break down both stored proteins and proteinaceous anti-nutrients in vegetable protein[75]. Regardless of dietary protein or energy concentrations, protease supplementation has been shown to improve the feed efficiency and digestibility of CP and fat and thereby reduce the nitrogen excretion[77]. Protease supplementation in diets improves the digestibility of protein and reduces the impact of anti-nutritional proteins on non-ruminant animals[11]. Yadav and Sah[4] added that the inclusion of dietary protease improves the digestibility of crude proteins in the diet and improves body weight gain in broilers. Furthermore, Rada *et al.*[78] reported that supplementation of microbial protease enzymes improved the carcass yield of broilers. Protease also has the capacity to improve amino acid digestibility and thereby improve the feed: body gain ratio of birds[77].

The digestibility of CP and starch increases in broilers fed diets supplemented with protease at most concentration[79]. However, each protease type has its own specificity and mode of action and hence the yield patterns of AA vary widely among the feedstuffs. Murugesan *et al.*[80] noted also that an increase in nutrient utilization when broiler diets were supplemented with protease and phytase (cocktail).

However, Cowieson and Ravindran[81] reported that there was no interaction between diets and enzyme products containing xylanase, amylase and protease in terms of ileal digestibility of nitrogen and Jiang *et al.*[82] reported that endogenous pancreatic proteases, e.g., trypsin and lipase, were not affected by supplementing exogenous amylase; rather, their intestinal activity was improved. Because phytic acid and NSP are not heat-labile, unlike some protease inhibitors and lectins, supplementing diets with exogenous phytase is very necessary. However, the efficacy of supplementation in chicken feeds depends mainly on the rate of inclusion rate as well as the age and genotypes of the birds. Feed enzymes are used to assist the breaking down of ANF in many feed ingredients for poultry and pigs[75] and generally, the pig and poultry industries are benefitting from the availability and use of phytase. Currently, around two-thirds of pig and poultry feeds contain supplemental phytase and this is yielding economic benefits[75]. Selle *et al.*[83] suggested, however, that vegetable proteins and cereal sources are different in their response to phytase supplementation in terms of amino acid digestibility.

CONCLUSION

The concentration of TI is almost absent in cereal grains; whereas highly concentrated in soybeans. Although , most ANF are suggested to be inactivated by heating, over- or under-heating still affects the quality. Breeding for low TI soybean cultivars currently has limited application as TI contributes a major proportion of sulfur containing AA. Although, TI caused pancreatic hypertrophy and increased secretions for chicks, rats and mice, others like pigs, dogs or pre-ruminant calves were free of these symptoms. When a processed soybean meal was increasingly replaced by raw soybean, the weights of pancreas and duodenum were proportionally increased. Supplementation of selected microbial feed enzymes could reduce the impacts of ANF and thereby improves the digestibility of proteins. As a recommendation it would have been suggested to use an optimum combination of heat intensity and time durations for improved quality of products while heating the soybean grains. Supplementation of selected and appropriate microbial feed enzymes is always advised to reduce both the heat-stable ANF and residual ANF in diets of non-ruminant animals.

REFERENCES

1. Bora, P., 2014. Anti-nutritional factors in foods and their effects. J. Acad. Ind. Res., 3: 285-290.
2. Pubols, M.H., 1990. Isolation, purification and the amino acid sequence of a secretory trypsin inhibitor from the chicken pancreas. Poult. Sci., 69: 640-646.
3. Ao, T., 2011. Using Exogenous Enzymes to Increase the Nutritional Value of Soybean Meal in Poultry Diet. In: Soybean and Nutrition, El-Shemy, H. (Ed.). InTech Publisher, Rijeka, Croatia, ISBN: 9789533075365, pp: 201-214.
4. Yadav, J.L. and R.A. Sah, 2005. Supplementation of corn-soybean broilers diets with different levels of acid protease. J. Inst. Agric. Anim. Sci., 26: 65-70.
5. Erdaw, M.M., M.M. Bhuiyan and P.A. Iji, 2016. Enhancing the nutritional value of soybeans for poultry through supplementation with new-generation feed enzymes. World's Poult. Sci. J., 72: 307-322.
6. Khokhar, S. and R.K.O. Apenten, 2003. Antinutritional Factors in Food Legumes and Effects of Processing. In: The Role of Food, Agriculture, Forestry and Fisheries in Human Nutrition, Khokhar, S. and R.K.O. Apenten (Eds.). UNESCO, Cambridge, pp: 82-116.
7. Natesh, H.N., L. Abbey and S.K. Asiedu, 2017. An overview of nutritional and antinutritional factors in green leafy vegetables. Horticult. Int. J., Vol. 1. 10.15406/hij.2017.01.00011.
8. Tacon, A.G.J., 1985. Morphological signs of nutrient deficiency and toxicity in farmed fish. Aquaculture Development and Coordination Program, Food and Agriculture Organization of the United Nations, Rome, Italy.
9. Francis, G., H.P.S. Makkar and K. Becker, 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197-227.
10. Ramadoss, B.R. and A.S. Shunmugam, 2014. Anti-dietetic factors in legumes-local methods to reduce them. Int. J. Food Nutr. Sci., 3: 84-89.
11. Pettersson, D. and K. Pontoppidan, 2013. Soybean Meal and the Potential for Upgrading its Feeding Value by Enzyme Supplementation. In: Soybean: Bio-Active Compounds, El-Shemy, A. (Ed.). Intech Open Access Publisher, Rijeka, Croatia, ISBN: 9789535109778, pp: 288-307.
12. Guillamon, E., M.M. Pedrosa, C. Burbano, C. Cuadrado, M.D.C. Sanchez and M. Muzquiz, 2008. The trypsin inhibitors present in seed of different grain legume species and cultivar. Food Chem., 107: 68-74.
13. Campbell, L.D. and F. Schoene, 1998. Effects of antinutritional factors in rapeseed: A review. Eur. Assoc. Anim. Prod., 93: 185-198.
14. Clemente, A., E. Jimenez, M.C. Marin-Manzano and L.A. Rubio, 2008. Active Bowman-Birk inhibitors survive gastrointestinal digestion at the terminal ileum of pigs fed chickpeabased diets. J. Sci. Food Agric., 88: 513-521.
15. Gu, C., H. Pan, Z. Sun and G. Qin, 2010. Effect of soybean variety on anti-nutritional factors content and growth performance and nutrients metabolism in rat. Int. J. Mol. Sci., 11: 1048-1056.
16. Sharma, S., R. Goyal and S. Barwal, 2013. Domestic processing effects on physicochemical, nutritional and anti-nutritional attributes in soybean (*Glycine max* L. Merill). Int. Food Res. J., 20: 3203-3209.
17. Van Eys, J., A. Offner and A. Bach, 2004. Manual of quality analysis for oybean products in the feed industry. US Soybean Export Council, pp: 1-113. https://www.slideshare.net/drvasuc/manual-of-quality-analysis-soya-products
18. Serrano, M.P., D.G. Valencia, J. Mendez and G.G. Mateos, 2012. Influence of feed form and source of soybean meal of the diet on growth performance of broilers from 1 to 42 days of age. 1. Floor pen study. Poult. Sci., 91: 2838-2844.
19. Crowell, G.L., 2012. Soybean meal-An exceptional protein source. Soybean Meal InfoCenter, Ankeny, IA.
20. Ravindran, V., M.R. Abdollahi and S.M. Bootwalla, 2014. Nutrient analysis, metabolizable energy and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci., 93: 2567-2577.

21. Ruiz, N., F. de Belalcazar and G.J. Diaz, 2004. Quality control parameters for commercial full-fat soybeans processed by two different methods and fed to broilers. J. Applied Poult. Res., 13: 443-450.
22. Sharma, S., M. Kaur, R. Goyal and B.S. Gill, 2014. Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. J. Food Sci. Technol., 51: 551-557.
23. Tacon, A.G.J., 1997. Fish Meal Replacers: Review of Antinutrients Within Oilseeds and Pulses-A Limiting Factor for The Aquafeed Green Revolution? In: Feeding Tomorrow's Fish, Cahiers Options Mediterraneennes, Volume 22, Tacon, A. and B. Basurco (Eds.). Institut Agronomique Mediterraneen de Zaragoza, Spain, pp: 153-182.
24. Bressani, R. and J.L. Sosa, 1990. Effect of processing on the nutritive value of Canavalia Jackbeans (*Canavalia ensiformis* (L.)). Plant Foods Hum. Nutr., 40: 207-214.
25. Liu, B.L., A. Rafiq, Y.M. Tzeng and A. Rob, 1998. The induction and characterization of phytase and beyond. Enzyme Microbiol. Technol., 22: 415-424.
26. Newkirk, R., 2010. Soybean: Feed Industry Guide. 1st Edn., Canadian International Grains Institute, Canada, Pages: 48.
27. Erdaw, M.M., R.A. Perez-Maldonado, M.M. Bhuiyan and P.A. Iji, 2015. Response of broiler chicks fed on steam-or cold-pelleted diets containing raw full-fat soybean meal. Proceedings of the 20th European Symposium on Poultry Nutrition, August 24-27, 2015, Prague, Czech Republic, pp: 179-180.
28. Erdaw, M.M., S. Wu and A.P. Iji, 2017. Growth and physiological responses of broiler chickens to diets containing raw, full-fat soybean and supplemented with a high-impact microbial protease. Asian-Aust. J. Anim. Sci., 30: 1303-1313.
29. Mogridge, J.L., TK. Smith and M.G. Sousadias, 1996. Effect of feeding raw soybeans on polyamine metabolism in chicks and the therapeutic effect of exogenous putrescine. J. Anim. Sci., 74: 1897-1904.
30. ASA., 2004. Whole soybeans in diets for poultry. American Soybean Association. http://www.thepoultrysite.com/articles/194/whole-soybeans-in-diets-for-poultry
31. Erdaw, M.M., R.A. Perez-Maldonado, M. Bhuiyan and P.A. Iji, 2016. Physicochemical properties and enzymatic *in vitro* nutrient digestibility of full-fat soybean meal. J. Food. Agric. Environ., 14: 85-91.
32. Emiola, I.A., A.D. Ologhobo and R.M. Gous, 2007. Performance and histological responses of internal organs of broiler chickens fed raw, dehulled and aqueous and dry-heated kidney bean meals. Poult. Sci., 86: 1234-1240.
33. Sarwar, G.G., X.C. Wu and K.A. Cockell, 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr., 108: S315-S332.
34. Hill, G.D., 2003. Plant Antinutritional Factors: Characteristics. In: Encyclopedia of Food Science and Nutrition, Caballero, B., L. Trugo and P.M. Finglas (Eds.). 2nd Edn., Academic Press, USA., ISBN: 9780122270550, pp: 4578-4587.
35. Denbow, D.M., 2000. Gastrointenstinal Anatomy and Physiology. In: Sturkie's Avian Physiology, Whittow, G.C. (Ed.). 5th Edn., Academic Press, UK., ISBN: 9780080542089, pp: 299-325.
36. Osman, A.M., 1982. Amylase in chicken intestine and pancreas. Compa. Biochem. Physiol. Part B: Compa. Biochem., 73: 571-574.
37. Shi, S.R., J. Lu, H.B. Tong, J.M. Zou and K.H. Wang, 2012. Effects of graded replacement of soybean meal by sunflower seed meal in laying hen diets on hen performance, egg quality, egg fatty acid composition and cholesterol content. J. Applied Poult. Res., 21: 367-374.
38. Erdaw, M.M., A.R. Perez-Maldonado, M. Bhuiyan and P.A. Iji, 2017. Partial replacement of commercial soybean meal with raw, full-fat soybean meal supplemented with varying levels of protease in diets of broiler chickens. S. Afr. J. Anim. Sci., 47: 61-71.
39. Erdaw, M.M., M.M. Bhuiyan and P.A. Iji, 2017. Response of broiler chicks to non-steam- or steam-pelleted diets containing raw, full-fat soybean meal. J. Applied Poult. Res., 26: 260-272.
40. Gemede, H.F. and N. Ratta, 2014. Antinutritional factors in plant foods: potential health benefits and adverse effects. Gobal Adv. Res. J. Food Sci. Technol., 3: 103-117.
41. Guillaume, J.C., S. Kaushik, P. Bergot and R. Metailler, 1999. Nutrition and Feeding of Fish and Shellfish. IFREMER, Paris, Pages: 489.
42. Liener, I.E., 1995. Possible adverse effects of soybean anticarcinogens. J. Nutr., 125: 744S-750S.
43. Karoly, D., 2011. Protease inhibitors. http://www.tankonyvtar.hu/en/tartalom/tamop425/0010_1A_Book_angol_04_takarmanyozastan/ch04s02.html
44. Clarke, E.J. and J. Wiseman, 2000. Developments in plant breeding for improved nutritional quality of soya beans II. Anti-nutritional factors. J. Agric. Sci., 134: 125-136.
45. Erdaw, M.M., R.A. Perez-Maldonado and P.A. Iji, 2017. Apparent and standardized ileal nutrient digestibility of broiler diets containing varying levels of raw full-fat soybean and microbial protease. J. Anim. Sci. Technol., Vol. 59. 10.1186/s40781-017-0148-2.
46. Logsdon, C.D. and B. Ji, 2013. The role of protein synthesis and digestive enzymes in acinar cell injury. Nat. Rev. Gastroenterol. Hematol., 10: 362-370.
47. Norton, G., 1991. Proteinase Inhibitors. In: Toxic Substances in Crop Plants, D'Mello, F.J.P., C.M. Duffus and J.H. Duffus (Eds.). The Royal Society of Chemistry, Cambridge, pp: 68-106.

48. Herkelman, K.L., G.L. Cromwell and T.S. Stahly, 1991. Effects of heating time and sodium metabisulfite on the nutritional value of full-fat soybeans for chicks. J. Anim. Sci., 69: 4477-4486.
49. Ruiz, N. and F. de Belalcazar, 2005. Field observation: Trypsin inhibitors in soybean meal are correlated with outbreaks of feed passage in broilers. Poult. Sci., 84: 70-70.
50. Ruiz, N., 2012. Soybean meal quality: Parameters beyond crude protein content in commercial Argentine SBM versus US SBM. Poult. Sci., 91: 86-86.
51. Susmel, P., M. Spanghero, S. Marchetti and S. Moscardini, 1995. Trypsin inhibitory activity of raw soya bean after incubation with rumen fluid. J. Sci. Food Agric., 67: 441-445.
52. Leontowicz, H., M. Leontowicz, H. Kostyra, G. Kulasek, M.A. Gralak, R. Krzeminski and M. Podgurniak, 2001. Effects of raw or extruded legume seeds on some functional and morphological gut parameters in rats. J. Anim. Feed Sci., 10: 168-183.
53. Banaszkiewicz, T., 2011. Nutritional Value of Soybean Meal. In: Soybean and Nutrition, El-Shemy, H. (Ed.). InTech Publisher, Croatia, ISBN: 978-953-307-536-5, pp: 1-20.
54. Clarke, E.J. and J. Wiseman, 2005. Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. Anim. Feed Sci. Technol., 121: 125-138.
55. Erdaw, M.M., R.A. Perez-Maldonado and P.A. Iji, 2018. Physiological and health-related response of broiler chickens fed diets containing raw, fullfat soya bean meal supplemented with microbial protease. J. Anim. Physiol. Anim. Nutr., 102: 533-544.
56. Rocha, C., J.F. Durau, L.N.E. Barrilli, F. Dahlke and P. Maiorka, 2014. The effect of raw and roasted soybeans on intestinal health, diet digestibility and pancreas weight of broilers. J. Applied Poult. Res., 23: 71-79.
57. Erdaw, M.M., R.A. Perez-Maldonado and P.A. Iji, 2018. Supplementation of broiler diets with high levels of microbial protease and phytase enables partial replacement of commercial soybean meal with raw, full-fat soybean. J. Anim. Physiol. Anim. Nutr., 102: 755-768.
58. Osborne, T.B. and L.B. Mendel, 1917. The use of soybeans as food. J. Biol. Chem., 32: 369-387.
59. Chernick, S.S., S. Lepkovsky and I.L. Chaikoff, 1948. A dietary factor regulating the enzyme content of the pancreas: Changes induced in size and proteolytic activity of the chick pancreas by the ingestion of raw soy-bean meal. Am. J. Physiol. Legacy Content, 155: 33-41.
60. Liener, I.E. and M.L. Kakade, 1980. Protease Inhibitors. In: Toxic Constituents of Plant Food Stuffs, Liener, I.E. (Ed.). 2nd Edn., Academic Press, New York, USA., ISBN-13: 9780124499607, pp: 7-71.
61. Grant, G., 1989. Anti-nutritional effects of soyabean: A review. Progr. Food Nutr. Sci., 13: 317-348.
62. Batterham, E.S., H.S. Saini, L.M. Andersen and R.D. Baigent, 1993. Tolerance of growing pigs to trypsin and chymotrypsin inhibitors in chickpeas (*Cicer arietinum*) and pigeonpeas (*Cajanus cajan*). J. Sci. Food Agric., 61: 211-216.
63. Struthers, B.J., J.R. MacDonald, R.R. Dahlgren and D.T. Hopkins, 1983. Effects on the monkey, pig and rat pancreas of soy products with varying levels of trypsin inhibitor and comparison with the administration of cholecystokinin. J. Nutr., 113: 86-97.
64. Flavin, D.F., 1982. The effects of soybean trypsin inhibitors on the pancreas of animals and man: A review. Vet. Hum. Toxicol., 24: 25-28.
65. Ge, Y.C. and R.G.H. Morgan, 1993. The effect of trypsin inhibitor on the pancreas and small intestine of mice. Br. J. Nutr., 70: 333-345.
66. Baba, N., T. Suzuki and T. Tobe, 1984. Effects of trypsin inhibitor on the remant pancreas after 85 percent pancreatectomy in rats. Jap. J. Surg., 14: 420-431.
67. Erdaw, M.M., 2018. Chickens' Response to Diets with Raw Soybean and Selected Feed Enzymes. Lab Lambert Academic Publishing, USA., Pages: 64.
68. Cowieson, A.J. and V. Ravindran, 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr., 98: 745-752.
69. Melmed, R.N. and I.A.D. Bouchier, 1969. A further physiological role for naturally occurring trypsin inhibitors: The evidence for a trophic stimulant of the pancreatic acinar cell. Gut, 10: 973-979.
70. Pusztai, A., 1998. Effects of Lectin Ingestion on Animal Growth and Internal Organs. In: Lectin Methods and Protocols, Rhodes, J.M. and J.D. Milton (Eds.). Humana Press, USA., pp: 485-494.
71. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.
72. Nahashon, S.N. and A.K. Kilonzo-Nthenge, 2013. Soybean in Monogastric Nutrition: Modifications to Add Value and Disease Prevention Properties. In: Soybean: Bio-Active Compounds, El-Shemy, H.A. (Ed.). In Tech Rijeka, Croatia, pp: 309-352.
73. Barth, C.A., B. Lunding, M. Schmitz and H. Hagemeister, 1993. Soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein in miniature pigs. J. Nutr., 123: 2195-2200.
74. Chen, L., P.V. Vadlani and R.L. Madl, 2014. High-efficiency removal of phytic acid in soy meal using two-stage temperature-induced *Aspergillus oryzae* solid-state fermentation. J. Sci. Food Agric., 94: 113-118.

75. Barletta, A., 2011. Introduction: Current Market and Expected Development. In: Enzymes in Farm Animal Nutrition, Bedford, M.R. and G.C. Partridge (Eds.). CAB International, Bodmin, UK., pp: 319-1-319-11.
76. Slominski, B.A., 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci., 90: 2013-2023.
77. Freitas, D.M., S.L. Vieira, C.R. Angel, A. Favero and A. Maiorka, 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. J. Applied Poult. Res., 20: 322-334.
78. Rada, V., M. Lichovnikova and M. Foltyn, 2014. The effect of serine protease on broiler growth and carcass quality. Acta Fytotech. Zootec., 17: 87-89.
79. Angel, C.R., W. Saylor, S.L. Vieira and N. Ward, 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poult. Sci., 90: 2281-2286.
80. Murugesan, G.R., L.F. Romero and M.E. Persia, 2014. Effects of protease, phytase and a *Bacillus* sp. direct-fed microbial on nutrient and energy digestibility, ileal brush border digestive enzyme activity and cecal short-chain fatty acid concentration in broiler chickens. PloS One, Vol. 9. 10.1371/journal.pone.0101888.
81. Cowieson, A.J. and V. Ravindran, 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci., 49: 37-44.
82. Jiang, Z., Y. Zhou, F. Lu, Z. Han and T. Wang, 2008. Effects of different levels of supplementary alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. Asian-Aust. J. Anim. Sci., 21: 97-102.
83. Selle, P.H., V. Ravindran, R.A. Caldwell and W.L. Bryden, 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev., 13: 255-278.