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EFFECT OF ISOFLAVONES ON GROWTH PERFORMANCE, DIGESTIBILITY, NITROGEN BALANCE AND CAECAL MICROBIAL ACTIVITY OF RABBITS

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Male New Zealand white rabbits were used to investigate the effect of two levels of isoflavones on the growth performance, nutrients digestibility, N-balance and caecal microbial activity. The experimental rabbits were randomly divided into three groups (n=5/group). The first group was used as a control, while groups 2 and 3 were given orally isoflavones at 2.5 (low dose, LD) and 5.0 (high dose, HD) mg/Kg body weight (BW), respectively. Doses of isoflavones were orally given to the animals every other day for 13 weeks.

The results showed that the LD (2.5 mg) of isoflavones increased feed intake, but there was no significant difference in water intake. Moreover, supplementing 2.5 mg of isoflavones improved the digestibility of all nutrients and total digestible nutrients (TDN) and digestible crude protein (DCP). On the other hand, isoflavones at 5.0 mg improved the digestibility of dry matter (DM), crude protein (CP) and ether extract (EE) only. Nitrogen balance increased by 27% in 2.5 mg- treated rabbits over the control. Supplementation of isoflavones slightly decreased the VFA with no effect on ammonia-N concentration in the caecum. This study concluded that isoflavones have a favorable effect on growth performance therefore, the animal، s diet must contain an adequate level of soybean meal, which covers the isoflavones requirements.
Keywords: Isoflavones, rabbits, performance, digestibility, caecum.

تأثير الأيزوفلافون على أداء النمو، معاملات الهضم، ميزان الأزوت، والنشاط الميكروبي في الأعور في الأرانب

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أجريت الدراسة علي ثلاث مجموعات من أرانب النيوريلندي الذكور بكل منها خمسة أرانب. المجموعة الأولي غذيت علي عليقة أساسية بدون إضافات ,المجموعة الثانية أعطيت ٢,٥ مجم /كجم من وزن الجسم عن طريق الفم من الأيزوفلافون, المجموعة الثالثة أعطيت ٥ مجم /كجم من وزن الجسم عن طريق الفم من الأيزوفلافون. جرعات الأيزوفلافون كانت تعطي يوم بعد يوم لمدة ١٣ أسبوع.

أظهرت النتائج زيادة في كمية العلف المأكولة في المجموعة الثانية فقط بينما لا يوجد فرق معنوي في كمية المياة بين المجاميع. كذلك تحسنت معاملات هضم جميع المكونات الغذائية والقيمة العذائية في المجموعة الثانية. علي الجانب الأخر وجد أن المجموعة الثالثة حدث تحسن فقط في معاملات هضم المادة الجافة, البروتين الخام والمستخلص الإيثيري فقط. تحسن ميزان الأزوت بحوالي ٢٧% في المجموعة الثانية مقارنة بالكنترول. حدوث إنخفاض طفيف في تركيز الأحماض الدهنية الطيارة مع عدم تأثر تركيز الأمونيا في الأعور. وتشير نتائج هذه الدراسة إلي أن للأيزوفلافون تأثير مرغوب علي أداء نمو الحيوانات وبالتالي يجب أن تحتوي العلائق علي مستوي مناسب من كسب فول الصويا لتغطية الإحتياجات من الأيزوفلافون.

INTRODUCTION

Soybeans are unique among beans because they contain compounds called isoflavones. Isoflavones are a specific type of phytochemical compounds. Isoflavones are found almost exclusively in legumes, the soybean and linseed in particular, provide the most abundant source of isoflavones and therefore most soya foods will provide a significant dietary source of these bioactive nonutrients (Reinli and Block, 1996). Isoflavones occur predominantly as glycosides in plants such as red clover and consequently are highly polar (water-soluble) compounds accounts for the low daily intake (Adams, 1995). Two isoflavone molecules, genistein and daidzein, have structures very similar to the body، s natural estrogens. Isoflavones are a type of phytoestrogens that are currently being studied for their potential health benefits. Phytoestrogens are compounds found in plants that act like weaker versions of the hormone estrogen. Estrogens are necessary for child bearing, sexual behavior and for bone and heart condition in humans. Isoflavones, genistein and daidzein, are found predominantly in soybeans and their products, mostly as their glycones (Reinli and Block, 1996). Because isoflavones are found almost exclusively in soybeans, mush of the recent soya investigation has focused on the role of isoflavones as potentially agents. Genistein and diadzein, the most significant isoflavones, are heterocyclic phenols that have a structure similar to estrogens. Subclinical and biological activity of phytoestrogens have been examined and confirmed in different animal species (Smith *et al*., 1979; Adams *et al*., 1988 and Kaldas and Hugh, 1989) but effects of such kind of compounds on rabbits and ruminants performance are likely to receive increasing attention. Therefore, the objectives of this study were to investigate the effect of supplementation of isoflavones on the growth, digestibility, nitrogen balance and ceacal microbial activity of rabbits.

MATERIALS AND METHODS

Animals and diet;

This study was carried out at the Department of the Bioscience and Technology, Institute of Graduate Studies and Research. Fifteen male New Zealand White rabbits (age of 6 months) were randomly allotted to three equal groups (n=5). The animals were individually housed in stainless steel cages. Group one served as a control, which animals were given a commercial pelleted ration (Table 1). However, groups 2 and 3 were given isoflavones at 2.5 (low dose LD) or 5.0 mg/kg body weight (high dose, HD), respectively. The dose of isoflavones was calculated according to the animal، s body weight on the week before dosing. The proper dose of isoflavones for each animal was filled into a gelatin capsule that was orally inserted with the help of a glass rod. The doses of isoflavones were chosen to be either 2.5 or 5 mg/kg BW according to Brzezinskim *et al*., (1997). The tested doses of isoflavones were given every other day for 13- week. Isoflavones (genistein/ daidzein from soya concentrate) were supplied in capsules (from California Health Products, Inc.11577W. Olympic Bivd., Los Angeles, and CA90064).

The diet was mixed well and supplied *ad libitum* once a day at 8:00 AM. In the following day feed residues were collected, weighted and stored for analysis. Feed intake was calculated by difference. All animals were weighted weekly to calculate the body weight gain.

Digestibility trials:

During the last week of the experimental period, the rabbits of each group, fed the same diet, were individually housed in metabolic cages for digestibility and nitrogen balance determinations according to Fekete (1985). These animals were kept for 7 days as a collection period. During this period, faeces and urine were quantitatively collected. Spot samples of fresh urine were stored frozen at – $10C^0$ for the analysis of total N. Faeces from each animal were pooled over the 7-d period for dry matter and chemical analysis. Caecal contents were removed immediately after slaughter and stored frozen at $-10C^0$ until analysis. The complete chemical composition of feed, feed residue, faeces and dry caecal contents were determined according to the AOAC (1990). Nitrogen was determined by micro- kjeldahl method. Total volatile fatty acids (VFA) in the caecal contents were estimated by steam distillation and titration (Warner, 1964). Ammonia-N was determined in the wet caecal samples by magnesium oxide methods (Markham, 1942).

Statistical analysis:

Data of the experiment for all variables were subjected to ANOVA as a completely randomized design according to Snedecor and Cochran (1982). The significant differences between treatment means were tested by the Duncan, s Multiple Range Test (Duncan, 1955).

Table 1. Determined proximate chemical analysis (%) of the experimental pelleted diet.

Composition	As fed	DM basis
Dry Matter	90.02	100.00
Organic Matter	82.16	91.27
Crude Protein	14.05	15.61
Crude Fiber	10.75	11.94
Ether Extract	03.16	03.51
Nitrogen Free Extract	49.75	60.21
ASH	07.86	08.73

RESULTS AND DISCUSSION

1- **Growth Performance:**

The effect of isoflavones on rabbits performance is given in Table (2). Average total gain and average daily gain (ADG) were 402 and 4.42 g for the control, 600 and 6.59g for the LD and 401 and 4.41g for the HD. The treatment did not improve the gain of animals during the experimental period. The reason for such slightly increase in ADG with LD might be due to the slightly higher feed intake accompanied by such treatment (Table 2). Contrariwise, the HD

caused a lower feed intake than in the control animals. However, its is known that the isoflavones are transformed by bacteria in the caecal flora during digestion, once this transformation has been done, the isoflavones exercise their beneficial effect in the body. Moreover, the list of biological properties associated with isoflavones is vast and includes both hormonal and non-hormonal actions (Kenneth *et al.*, 1998). Also, isoflavones are protective antioxidants which reduce the formation of free radicals and reactive oxygen by decomposition of hydrogen peroxide without generating free radicals, by quenching active single oxygen, and by trapping and quenching radicals before they reach a cellular target (Fran *et al*., 2000).

Table 2: Growth performance of rabbits as influenced by feeding diet with or without isoflavones

Items	Control	LD	HD
No. of animals	5	5	5
Initial body weight (kg)	2.900	2.600	2.860
Final body weight (kg)	3.302	3.200	3.261
Experimental period, (d)	91	91	91
Feed intake (g/d)	173.96	187.74	161.41
Water intake, (ml/d)	500.3	508.3	529.5

2- Digestibility and feeding value:

The digestibility of nutrients and nutritive value are presented in Table (3). The data showed that adding of LD of isoflavones improved significantly ($P<0.05$) the digestibility of DM, OM, CP, EE and NFE. While, the HD of isoflavones significantly ($P<0.05$) increased the digestibility of EE only. The increase of digestion coefficients led to an improvement in the nutritive value of total digestible nutrients (TDN) and digestible crude protein (DCP) being 68.92 and 19.46 for the control, 76.59 and 23.67 for the LD and 70.89 and 19.0% for the HD, respectively. The values of nutritive ratio (NR) in treated animals were decreased due to the improvement of DCP.

The treatment with isoflavones caused a significant ($P< 0.01$) decrease in the levels of free radical in plasma, testes, liver and brain (data not shown). The protective effect of isoflavones from the negative (toxic) effect of free radicals on cell membrane and DNA of the microorganisms in the caecum may be the reason of improved digestion function. Carroll, 1991 and Anderson *et al*. 1995 found that consumption of soya protein (in replace of animal protein) leads to a decrease in the concentration of serum cholesterol. Also, treated rabbits with isoflavones showed significant ($P<0.01$) decline in the concentration of plasma cholesterol and low density lipoprotein (LDL) (data not shown). These are a good indicator and coincided with the improvement of EE digestion (Table 3). This might be ascribed to the availability of cholesterol as a low-density lipoprotein to different tissues. This is supported in form of a slight increase in animal weight of the treated animals (Table 2).

3- Nitrogen balance and caecal microbial activity:

The treatment of rabbits with isoflavones decreased the N output (faeces and urine) by about 21% (Table 4). The improved N balance at the LD of isoflavones was coinceded with highest daily gain. When N balance was adjusted to the nitrogen intake, the improvement went up to 17.7 and 8.77 % for LD and HD, respectively. Isoflavones has been shown to decrease cortisol

levels and increase protein utilization (Correll, 1991). The administration of isoflavones decreased the total Volatile Fatty Acids (VFA) concentration but slightly increased the NH3-N concentration (Table 4). This could be attributed to a direct stimulatory influence of isoflavones on the caecal bacteria, and /or the protective effect against the harmful effect of free radical. Borriello *et al*. (1985) showed almost two decades age that intestinal microflora play a key role in the metabolism and bioavailability of isoflavones. After ingestion, soybean isoflavones are hydrolysed by intestinal glucosidases which release the aglycones, daidzein, genistein and glycitein. These may be absorbed or further metabolized to many specific metabolites including, equol and p-ethyl phenol (Joannou *et al*.,1995). The extent of this metabolism appears to be highly variable among individuals and is influenced by other components of the diet (Kenneth *et al*.,1999). A high carbohydrate milieu, which causes increased intestinal fermentation, result in more extensive biotransformation of phytestrogens, with greatly increased formation of equol, a mammalian isoflavones metabolite of daidzein. This metabolic pathway may be clinically relevant to the efficacy of soybean isoflavones because the esrtogenic potency of equol is an order of magnitude higher than its precursor, daidazen (Kenneth *et al*., 1999). The important of the microflora in the metabolic handling of phytoestrogens is illustrated from observation that antibiotic administration block metabolism, germfree animals do not excrete the metabolites and infants fed soy infant formulas in the first 4 months of life, when gut microflora are underdeveloped, cannot from appreciable amounts of equol (Setchell *et al*.,1998).

Table 3. Effect of isoflavones on digestion coefficient and nutritive value in male rabbit.

Items	Control	LD	HD
Nutrient digestibility, %			
DM	71.26[a]	79.81[b]	71.20 [a]
OM	72.01[a]	79.94 [b]	73.71 [a]
CP	71.66[a]	80.77 [b]	75.38 [a]
CF	51.08 [a]	62.36 [a]	54.99 [a]
EE	88.87 [a]	93.88 [b]	94.89 [b]
NFE	74.91 [a]	82.25 [b]	75.43 [a]
Nutritive value, %			
TDN	68.92 [a]	76.59 [b]	70.89 [a]
DCP	19.46 [a]	23.67 [b]	19.00 [a]
NR, g/h/d	2.73	2.24	2.54

a-b: Means in the same row followed by different superscripts differ significantly (P<0.05).

Table 4. Effect of isoflavones on nitrogen balance and caecal activity of male rabbit.

Item	Control	LD	HD
Nitrogen (N) balance, g/d			
N intake	4.35	4.69	4.03
N in feces	1.23	0.90	0.99

N in urine	0.48	0.44	0.38
Total N out -put	1.71	1.34	1.37
N balance	2.64	3.35	2.66
Improvement, %	-	27 %	-
N balance/N intake, %	60.69	71.43	66.01
Improvement, %	-	17.70	8.77
Microbial activity			
NH3- N (mg/ 100g)	15.44 [a]	16.35 [a]	15.61 [a]
VFA (mmol /100g)	14.92 [a]	13.19 [a]	12.80 [a]

a: Means within rows followed by a common superscript do not differ (P>0.05).

CONCLUSION

In conclusion, this study suggested that animal's diets should contain an adequate level from soybean to cover the requirements of isoflavones, which play an important role in animal growth and performance. A soy-based diet has been reported to cause estrogenic effects in swine (Drane *et al*., 1981) and laboratory animals (Sharma *et al*., 1992), but there are no reports of effects in ruminants therefore, we recommend that the roles of isoflavones in ruminants should be examine.

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