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Effects of dietary inclusion of alfalfa (*Medicago sativa* L.) leaf meal and Xylam enzyme on laying hens' performance and egg quality

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Abstract

The experiment examined the effect of inclusion of sun-dried alfalfa leaf meal and 0.05 Xylam enzyme (ALM+X) in laying hens' diet on egg production and quality. Forty eight White Hisex laying hens aged 20 weeks were offered four iso-caloric and iso-nitrogenous diets (D_1, D_2, D_3 and D_4 of 0.0, 2, 5 and 7% ALM, respectively) supplemented with Xylam enzyme (0.05%), for 8 weeks. Supplementation of ALM+X to layer hens' diet showed insignificant differences in feed consumption, feed conversion ratio, hen-day egg production percentage, egg weight, egg albumin height and egg specific gravity when compared to alfalfa free diet. It, however, improved eggshell thickness, egg yolk index and egg yolk colour and reduced egg yolk cholesterol. It was concluded that addition of exogenous enzymes to layer hen diet containing alfalfa overcome adverse effects of alfalfa on performance parameters and egg quality characteristics.

Key words: Alfalfa Leaf Meal, Cholesterol, Enzyme, Layers.

Introduction

Alfalfa is available as major forage for animals in Sudan. Dehydrated alfalfa has been reported to be quite high in protein and fibre and improve pigmentation of the skin and egg yolk (Fletcher and Papa, 1985; Leeson and Summers, 2008). Mourão et al. (2006) suggested that due to the higher content of fibre in alfalfa, its use in monogastric animals should be restricted. In addition, it is reported that, alfalfa leaf meal (ALM) reduced serum cholesterol of laying hens (Wen-jun et al., 2007), lowered egg yolk cholesterol and increased eggshell thickness (Güçlü et al., 2004). The reduction of egg yolk cholesterol as a result of addition of ALM to laying hen diet is due to the presence of saponins (Sidhu and Oakenfull, 1986). Whitehead et al. (1981) suggested that reduction in feed consumption, body weight and egg production was due to the supplementation of ingredient with high saponins content to laying hens. In addition, Mourão et al. (2006) mentioned that supplementation of laying hens diet with alfalfa reduced feed intake, egg weight, egg production and egg mass. Alfalfa has some beneficial effects on poultry production. Increased fibre content of alfalfa restricts its use in monogastric animals, specifically poultry sector. To improve the ability of birds to digest fibres, enzymes are added, to increase energy utilization and overcome the negative effects of fibres on gut lumen activity and excreta consistency (Leeson and Summers, 2008). Nutrase Xylam 500 is a bacterial enzyme preparation, which contains endo-xylanase and α-amylase. Ritz et al. (1995) and Santos Jr. et al. (2004 a & b) reported that addition of xylanase enzyme to male turkey diet enhanced weight gain, feed conversion ratio (FCR) and feed consumption. According to Odetallah (2000), amylase and lipase are used in poultry to improve nutrients digestibility and performance characteristics.

This study was performed to evaluate the role of Xylam enzyme in overcoming the adverse effects of dietary ALM inclusion on laying hen performance and egg quality characteristics.

Materials and Methods

Fresh Alfalfa leaves were bought from Alhalfayah Market in Khartoum suburb. Fresh Alfalfa leaves were distributed evenly on the floor of an open shady place

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in poultry house, turned up-side down once or twice a day. The collected dried Alfalfa leaves were ground into homogenously reasonable size using a hammer mill. Alfalfa leaves were chemically analyzed for dry matter, crude protein, crude fibre, ether extract and ash contents (Table 1) according to AOAC (1990).

Forty eight White Hisex laying hens about 20 weeks old were purchased from Coral Hatcheries. All required practices (vaccination for Gumboro, Newcastle and Fowl Pox) were carried out during the brooding and rearing periods as recommended. After arrival at the experimental house, all hens were fed on the diet recommended by NRC (1994). Birds were randomly distributed in 16 cages of a battery, placed in an open sided house. Three birds were allotted in each small cage that represents a replicate. The batteries were provided with longitudinal feeders and automatic drinkers. Light was provided for 15-17 hours in the form of natural day light supplemented with artificial light in the evening by using bulb lambs of 60 watt above the batteries. All possible steps were taken to avoid animal suffering at each stage of the experiment.

Four experimental iso-caloric and iso-nitrogenous diets (D_1, D_2, D_3 and D_4 of 0.0, 2, 5 and 7% of ALM, respectively and 0.05 % Xylam) were formulated. The experimental diets were formulated (Table 2) to meet the laying hen requirements according to NRC (1994). Each experimental diet was assigned randomly to four replicates. Table 3 shows the calculated chemical analysis of the layer experimental diets. The experimental diets and water were offered *ad libitum.*

The birds under study had approximately equal weight and were subjected to adaptation to the batteries before data collection for a period of one week during which the birds were fed commercial diets. Clean fresh water and feed were offered *ad libitum*. During the experimental period the produced eggs per replicate were weighed daily and computed for Hen-day egg production percentage, egg weight, and feed conversion ratio. At the end of every two weeks, eight eggs (2 eggs from each replicate) for each treatment were randomly selected for examination of egg quality characteristics (egg weight, egg length, egg diameter, egg shell thickness, yolk diameter, yolk height, albumin height and egg yolk colour). The egg dimensions (length, diameter, shell thickness, and heights) were measured in millimetres (mm) using Digital Vernier Calliper. Egg yolk colour was measured using Yolk Colour Fan. During the last week of the experiment, two eggs from each replicate (8 eggs per treatment) were randomly selected and marked to be used to examine the content of egg yolk cholesterol.

Statistical analyses

The experiment was conducted in a complete randomized design. Analysis of variance (ANOVA) as described by Steel and Torrie (1980) was performed on all data using the General Linear Models procedure of SAS (1990). Differences between dietary treatment means were tested using Duncan's Multiple Range Tests (Duncan, 1955).

Results

No significant difference was found for feed intake among the control and treated birds during the entire

Table 1: Chemical composition of dried alfalfa leaves

Item	Percent
Dry Matter	96.1
Crude Protein	22.75
Ether Extract	1.14
Crude Fibre	13.26
Ash	5.81
Nitrogen Free Extract	53.14
Calculated Metabolizable Energy (Kcal/Kg)	2300

Table 2: Ingredients percentages of the experimental laying hens' diets (on fed basis)

Ingredients (%)	D_1	D_2	D_3	D_4
Alfalfa Leaf Meal	0.0	2	5	7
Sorghum Grain	70.00	70.00	69.90	68.42
Ground Nut Meal	11.60	11.30	10.62	10.00
Wheat Bran	3.97	2.22	0.00	0.00
Limestone	8.10	8.10	8.10	8.10
Layer Concentrate*	5.00	5.00	5.00	5.00
Di-Cal. Phosphate	0.10	0.10	0.10	0.10
Salt (NaCl)	0.20	0.20	0.20	0.20
Choline	0.05	0.05	0.05	0.05
Anti toxin	0.20	0.20	0.20	0.20
Organic acid	0.20	0.20	0.20	0.20
Lysine	0.31	0.31	0.31	0.31
Methionine	0.17	0.17	0.17	0.17
Vegetable Oil	0.05	0.10	0.10	0.20
Xylam	0.05	0.05	0.05	0.05
Total	100	100	100	100

Hendrix * Crude protein 40% , Crude fat 3% , Crude fiber 2% , Calcium 6% , Phosphorous (available) 5.8%, Lysine 6% , Methionine 2.8% , Methionine + Cystine 3.3% , Sodium 1.6%, ME (Kcal/kg) 2000, Vitamins: Vit. A 240.000 I.U/kg, Vit. D3 60.000 I.U/kg, Vit. E 800 mg/kg, Vit. K3 40 mg/kg, Vit. B1 30 mg/kg, Vit. B2 100 mg/kg, Vit B6 50 mg/kg, Vit. B12 400 mg/kg, D-pantothenic acid 130 mg/kg, Niacine 700 mg/kg, Antioxidant (BHT) 900 mg/kg, Choline chloride 8000 mg/kg, Folic acid 10 mg/kg, Manganese 1810 mg/kg, Zinc 1080 mg/kg, Iron 1000 mg/kg, Copper 151 mg/kg, Iodine 20 mg/kg, Selenium 5 mg/kg and Cobalt 20 mg/kg, Xanthophyll added, phytase added

Table 3: Nutrients' composition of the experimental laying hens' diets (on dry matter basis)

Diet treatment	Nutrients						
	ME (Kcal/Kg)	CP%	Lys %	Meth %	Ca%	P%	Na%	CF%
D_1	2806	17.48	0.86	0.44	3.37	0.41	0.17	3.42
D_2	2810	17.49	0.85	0.43	3.37	0.40	0.17	3.17
D_3	2813	17.45	0.83	0.42	3.37	0.40	0.83	2.81
D_4	2802	17.39	0.82	0.42	3.37	0.39	0.17	2.71

D1: 0% ALM+0.05% Xylam; D2: 2% ALM+0.05% Xylam; D3: 5% ALM+0.05% Xylam; D4: 7% ALM+0.05% Xylam

Table 4: Feed intake (g/bird/day) of laying hens of the experimental diets groups during the experimental period

	Weeks							
	1st	2nd	3rd	4th	5th	6 th	7th	8th
D_1	98.57±13.00^a	97.68±8.77^a	96.37±8.7 ^{a}	103.1±7.25^a	103.5±8.08^a	106.4±6.65^a	112.9±7.76^a	111.1±12.54^a
D_2	83.16±12.35^a	92.44±5.10^a	91.43±7.01^a	99.05±6.69^a	100.4±3.72^a	103.8±4.12^a	113.3±0.99^a	112.1 ±2.49^a
D_3	85.78±12.01^a	101.0±8.26^a	99.58±6.32^a	103.6±12.59^a	103.9±8.48^a	107.4±7.18^a	111.3±9.04^a	114.1 ±8.06^a
D_4	92.62±7.43^a	98.10±7.41^a	99.53±10.95^a	107.4±7.67^a	107.4±9.06^a	111.0±6.07^a	115.9±4.43^a	114.6 ±6.91^a

D1: 0% ALM+0.05% Xylam; D2: 2% ALM+0.05% Xylam; D3: 5% ALM+0.05% Xylam; D4: 7% ALM+0.05% Xylam;
$^{a-c}$Means in the same column with different superscripts are significantly different (P<0.05)

Table 5: Hen-day egg production percentage of laying hens of the experimental diets groups during the experimental period

D_n	Weeks							
	1st	2nd	3rd	4th	5th	6th	7th	8th
D_1	72.62±7.15^{ab}	90.48±16.0^a	97.62±2.75^a	97.62±2.75^a	97.62±4.76^a	100.0±0.00^a	95.24±3.89^a	97.62 ±4.76^a
D_2	65.48±21.78^{ab}	88.10±12.60^a	84.52±17.11^a	96.43±7.15^a	95.24±6.74^a	94.05±2.38^a	101.2±2.38^a	101.2±2.38^a
D_3	86.91±11.90^a	88.10±9.12^a	94.05±4.56^a	97.62±4.76^a	98.81±4.56^a	100.0±3.89^a	95.24±6.74^a	98.81 ±2.38^a
D_4	64.29±6.15^b	91.67±10.56^a	91.67±9.01^a	95.24±6.74^a	100.0±3.89^a	104.8±12.90^a	98.81±2.38^a	101.2 ±2.38^a

Table 6: Weight of egg (g) of laying hens of the experimental diets groups during the experimental period

	Weeks							
	1st	2nd	3rd	4th	5th	6th	7th	8th
D_1	43.66±1.63 ^{ab}	47.69±1.84^a	51.65±1.24^a	52.33±1.01^a	53.85±1.53^a	54.88±1.38 ^{a}	55.93±0.56^a	56.33±2.43^a
D_2	40.51±1.66^b	44.66±1.39^b	48.48±2.15^a	50.47±2.23^a	51.35±2.33^a	53.58±2.49^a	53.86±2.31^a	55.43 ±2.89^a
D_3	42.58±2.44^{ab}	46.56±2.43^{ab}	49.37±2.70^a	51.70±2.31^a	52.06±1.83^a	54.25±2.46^a	54.24±1.87^a	54.91 ±2.47^a
D_4	44.64±1.95^a	46.53±1.46^{ab}	50.95±2.59^a	51.78±1.58^a	53.91±1.42^a	55.75±1.85^a	55.46±1.38^a	56.22 ±2.08^a

Table 7: Feed Conversion Ratio (g feed/g egg) of laying hens of the experimental diets groups during the experimental period

	Weeks							
	1st	2nd	3rd	4th	5th	6th	7th	8th
D_1	3.14±0.59^{ab}	2.33±0.50^a	1.91±0.12^b	2.02±0.17^a	1.98±0.28^a	1.94±0.16^a	2.12±0.20^a	2.02 ±0.09^a
D_2	3.33±0.79^a	2.39±0.38^a	2.29±0.38^a	2.04±0.09^a	2.06±0.17^a	2.06±0.05^a	2.08±0.14^a	2.01±0.14^a
D_3	2.33±0.20^b	2.48±0.27^a	2.15±0.09^{ab}	2.05±0.08^a	2.02±0.14 ^{a}	1.98±0.04^a	2.16±0.19^a	2.10 ±0.08^a
D_4	3.26±0.45^a	2.32±0.28^a	2.14±0.17^{ab}	2.18±0.05^a	1.99±0.13^a	1.92±0.23^a	2.12±0.05^a	2.02 ±0.03^a

Table 8: Shape index of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	75.14±2.16^a	76.12±1.63^a	75.79±1.07^a	76.04±2.35^a
D_2	75.41±2.82^a	75.69±3.36^a	76.76±1.59^a	76.06±2.59^a
D_3	75.68±1.29^a	75.62±2.09^a	76.09±1.37^a	76.33±2.21^a
D_4	75.69±2.77^a	76.80±2.36^a	76.62±3.07^a	76.90±2.71^a

Table 9: Shell thickness (mm) of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	0.26 ±0.07^b	0.36±0.04^b	0.38±0.02^b	0.38±0.01^b
D_2	0.37±0.01^a	0.36±0.02^b	0.36±0.02^b	0.41±0.01^a
D_3	0.40±0.02^a	0.37±0.02^{ab}	0.38±0.01^b	0.40±0.02^a
D_4	0.39±0.01^a	0.38±0.01^a	0.43 ±0.03^a	0.41±0.01^a

Table 10: Albumen Height (mm) of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	6.58±1.51^a	7.17±0.83^a	6.07±0.98^a	7.62±1.04^a
D_2	6.94 ±0.51^a	6.57 ±0.98^a	6.37±1.02^a	7.10±1.01^a
D_3	6.58±0.83^a	6.55±0.65^a	6.08±1.10^a	7.17±1.01^a
D_4	6.64±0.44^a	6.55±0.57^a	6.28±1.08^a	7.60±1.08^a

Table 11: Haugh Unit of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	20.75±2.94[a]	22.16±1.64[a]	20.51±2.01[a]	22.72±2.24[a]
D_2	21.70±0.88[a]	21.00±1.94[a]	20.28±2.10[a]	21.78±1.90[a]
D_3	20.90±1.61[a]	20.92±1.29[a]	19.66±2.27[a]	21.98±1.92[a]
D_4	21.07±0.96[a]	20.90±1.23[a]	19.91±2.19[a]	22.58±2.11[a]

Table 12: Yolk index of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	40.20±3.65[b]	42.88±2.21[b]	45.97±1.38[a]	43.03±2.10[b]
D_2	42.97±0.76[a]	45.78±1.19[a]	44.37±1.84[a]	47.70±2.41[a]
D_3	43.50±1.04[a]	45.02±2.72[a]	44.95±1.76[a]	46.11±2.09[a]
D_4	41.69±2.31[ab]	44.34±1.33[a]	45.67±2.57[a]	48.31±2.43[a]

Table 13: Yolk colour of eggs of laying hens of the experimental diets groups during the experimental period

	Weeks			
	2nd	4th	6th	8th
D_1	4.13±0.35[c]	4.00±0.53[d]	4.13±0.83[b]	5.50±0.76[c]
D_2	8.50±0.53[b]	7.25±0.71[c]	8.13±0.35[a]	8.75±0.46[b]
D_3	8.25±0.46[b]	8.38±0.52[b]	8.50±1.07[a]	8.75±0.46[b]
D_4	9.75±0.89[a]	9.25±1.28[a]	8.50±0.53[a]	9.88±0.35[a]

Table 14: Yolk cholesterol of eggs of laying hens of the experimental diets groups

	Egg Yolk Cholesterol (unit?).
D_1	12.87±0.84[a]
D_2	10.51±1.51[c]
D_3	12.02±0.77[ab]
D_4	11.32±0.82[bc]

[a-c]Means in the same column with different superscripts are significantly different (P<0.05)

experimental period (Table 4). Egg production was significantly high in first week in D_3 compared to D_4 (Table 5). However, egg weight was significantly higher in D_4 compared to D_2 (Table 6). The FCR was significantly low in first week in D_3 and in 3rd week in D_1 (Table 7). No significant effect of dietary treatment on egg shape index was observed (Table 8). Egg shell thickness was significantly high in D_4 (Table 9). No significant difference was found for egg albumin height and egg specific gravity between the control and treated groups as shown in Tables 10 and 11. As shown in Tables 12 and 13, egg yolk index and yolk colour were significantly high in D_4 while egg cholesterol concentration was significantly low in all the treated groups compared to control (Table 14).

Discussion

The findings of the chemical analysis of alfalfa are not in agreement with the results observed by ElFaki (2009) who reported the proximate analysis of alfalfa meal to be 17.5% for crude protein, 26.65% for crude fibre, 1.65% for ether extract and 8.91% for ash. This disagreement may be due to the difference in stage of maturity of the collected leaves, soil and environmental conditions (Aganga and Omphile, 2005).

The current results indicated that addition of Xylam enzyme restored the negative effect of ALM on feed consumption, FCR, egg weight and percentage of hen-day egg production percentage which were reported to be deteriorated by feeding alfalfa (Whitehead et al., 1981; Mouräo et al., 2006; Mouräo et al. 2006). These findings agreed with those of Lazaro et al. (2003) who found that addition of polysaccharidases to cereal-based diet (wheat, rye or barley) of laying hens increased egg production and improved feed efficiency.

Eggshell thickness increased due the addition of Xylam to the diets containing 5 or 7% ALM compared to control and 2% ALM diet. The current results are inconsistent with the findings of Mouräo et al. (2006) and Khajali et al. (2007) who reported that addition of alfalfa and enzyme to laying hens diet had no effect on eggshell thickness. Improvement of egg yolk colour as a result of supplementation of Xylam to laying hens' diet containing different levels of alfalfa in this study was in line with the findings of Çiftci et al. (2003) who mentioned that supplementation of triticale and wheat triticale-based diets with enzyme significantly improved egg yolk colour. Mouräo et al. (2006) and Khajali et al. (2007) found contradictory results. They observed the absence of effect of the enzyme when supplied to a diet containing alfalfa on egg yolk colour. This contradiction may be due to the use of higher level of alfalfa, difference in enzymes types and age of the birds. Bedford (1995) reported that addition of enzymes to the diet of older laying hens had little effects on egg production. Reduction in egg yolk cholesterol due to addition of Xylam to diet containing 2% alfalfa was in agreement with Khajali et al. (2007) who mentioned that exogenous enzyme reduced egg yolk cholesterol when used in laying hens' diet containing alfalfa.

Conclusion

It was concluded that supplementation of laying hen diet containing alfalfa with exogenous enzyme (Xylam) improved egg quality characteristics, especially egg yolk colour and yolk cholesterol.

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