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Response of Growing Buffalo Calves to Dietary Supplementation of Caraway and Garlic as Natural Additives

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Abstract: The current study was conducted to examine the ability of using natural additives to manipulate rumen fermentation, improve nutrient utilization and animal performance in growing male buffalo calves. Sixteen buffalo calves of 12-14 months of age weighing 170 ±9.6 kg were divided into 4 groups, 4 animals / each. Caraway seed and garlic powder were added to calves basic diet (control) at levels of 2 g caraway seed (CS,T_1), 2 g dried garlic (DG,T_2), 2 g caraway and 2 g garlic (T_3) / kg diet and were fed for 6 months experimental period. Blood metabolites were monthly determined. Before the end of the experiment a digestibility trail was performed and rumen liquor was collected. Daily feed consumption was recorded. The results showed that feeding caraway garlic, or both significantly increased ($p<0.05$) nutrient digestibilties (DM, CP, EE, CF), however NFE and OM digestibilties were similar among treatment groups. Dry matter intake, Final weight, weight gain and feed conversion efficiency were slightly improved ($p>0.05$) in calves fed both caraway and garlic (T_3) than other groups. In addition, caraway and garlic improve rumen fermentation parameters in terms of increasing total volatile fatty acids, increasing rumen pH and decreasing rumen ammonia. Moreover, calves fed the treatment diets had greater ($P < 0.05$) serum concentrations of total protein, globulin and lower cholesterol and ALT compared with those fed the control diet. The findings of the present study suggest that caraway and garlic can be supplemented to growing male buffalo for better nutrient digestibilties, performance, rumen fermentation and metabolic status.

Key words: Growing Buffalo Calves · Dried Garlic (DG) · Caraway Seed (CS) · Performance · Dietary Supplementation · Nutrient Digestibility

INTRODUCTION

Buffaloes are the prime source of good quality meat and milk in Egypt and some other developing countries. These animals are mainly reared in small holder farms and suffer from a lot of stressful conditions such as mal-nutrition and characterized by inferior productive and reproductive potentials [1].

Feed additives are important materials that can improve the efficiency of feed utilization and animal performance. Modern animal production requires the use of safe and effective additives to stimulate feed consumption and destroy harmful microorganisms of the diet. Attempt to use natural materials such as medicinal plants are widely accepted as feed additives [2].

World Health Organization encourages using of medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to the nature. Also, the use of antibiotics as feed additives in dairy or growing animals has been of increasing concern due to the potential appearance of residues in milk and meat. Furthermore, the use of antibiotics as a feed additive has been banned in the European Union [3]. For this reason, scientists are interested in evaluating the potential use of natural antimicrobials such as herbs and plant extracts. Currently, the use of plant herbs has resulted in improving rumen ecology [4, 5] supporting performance and health status [6].

Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation

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of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract [7] and had the highest stimulatory influence particularly on bile secretion and pancreatic enzymes activity [8].

Garlic supplementation through feed in particular has many favorable experimental and clinical effects which include stimulation of immune function, enhanced foreign compound detoxification, restoration of physical strength and resistance to various stresses [9]. The primary sulphur containing constituents in whole, intact garlic are cysteine sulfoxides [10]. Additional constituents of intact garlic include steroidal glycosides [11], lectins [12] prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B-1, B-2, B-6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and amino acids [13]. Caraway (*Carum carvi*) seed is used in meat, food and distillery industries due to its pleasant flavor and intense taste. Its antibacterial and fungicidal properties are important in pharmaceutical applications and also in human and veterinary medicine [14].

The pharmacological action of active plant substances or herbal extracts in humans is well known, but in animal nutrition the number of precise experiments is relatively low. Therefore, the study was conducted to investigate the effects of feeding garlic, caraway or both on performance body weight gain, feed intake, feed conversion, nutrient digestibility and blood metabolites of growing buffalo calves..

MATERIALS AND METHODS

Animals, Diets and Management: The experiment was carried out at the research farm of Faculty of Agriculture, Al-Azhar University Assiut, Egypt. Sixteen male buffalo calves of similar age 12-14 months and body weight (170 ±30.57 kg) were randomly assigned into four groups. The calves fed four dietary treatments. The experimental diets consisted of an un-supplemented control diet based on concentrate mixture, wheat straw and green Berseem (*Egyptian clover*) or similar diets supplemented with 2 g caraway seed powder (CS) / kg diet (T_1) or 2 g dried garlic powder (DG) /kg diet (T_2) or both (2 g CS plus 2 g DG /kg diet, T3). So the dietary treatments were as follows:

Table 1: Ingredients composition (%) of concentrate mixture of experimental diets

Item	Control	T1	T2	T3
Yellow corn	50	50	50	50
Wheat bran	27.2	27	27	26.8
Cotton seed cake	20	20	20	20
Limestone	2	2	2	2
Premix*	0.3	0.3	0.3	0.3
Salt	0.5	0.5	0.5	0.5
Garlic	0	0.2	0	0.2
Caraway	0	0	0.2	0.2
Total	100	100	100	100

*Compound premix each 3 kg contain: 1,250,000 IU, Vit A; 2,500,000 IU, Vit D3; 1000 mg, Vit E; 80,000 mg, Mn; 60,000 mg, Zn; 50,000 iron, 20,000 copper, 5000 iodine, 250 Se, 1000 Co mg tell 3 kg CaCO3

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

control (basal diet), T1 (basal diet + 2g CS /kg), T2 (basal diet + 2 g DG /kg) and T3 (basal diet + 2 g CS plus 2 g DG /kg). The ingredients and chemical composition of experimental diets are shown in Table 1 and 2. Diets were mixed daily and fed twice a day. Feed intake was recorded daily and their representative samples were taken for chemical analysis. Calves were housed on concrete-floor in separate pens. They were fed individually in locally manufactured mangers. The calves were weighed initially and every month thereafter. Economic analysis of data was done using the technique of Perrin *et al*. [15]. Round the clock fresh and clean water was available to them. Calves were de-wormed at the start of the experiment. The study lasted for 6 months.

Digestion Trial: Digestion of the feed components was carried out using chromic oxide as indicator. The digestion trial lasted 5-days as preliminary period and 7-day as collection period. The total rations contained approximately 0.5 % Cr2O3 which has been mixed with the concentrate portion. The rations were sampled daily during the trial and the samples were composited at the end of each trial, ground through 1 ml. screen and stored for chemical analysis. Fecal grab samples containing approximately 200 g of fresh feces were taken twice during each 24-hr. period and composited by animal at the end of the 7-day collection period. Feces were mixed thoroughly and 20% of it was sampled and dried at 55°C in forced air oven. The rations and fecal samples were analyzed for Cr_2O_3, dry matter (DM), crude protein (CP), crud fiber (CF), ether extract (EE) and ash.

Table 2: Chemical composition of experimental diets (% as fed)

	DM	OM	CP	CF	EE	NFE	Ash	TDN	ME*	Ca	P
Control	90.73	86.27	15	19.98	3.91	47.39	4.46	66.32	2.38	0.9	0.6
T_1	90.14	84.87	15	20.05	4.61	45.21	5.27	66.32	2.38	0.9	0.6
T_2	91.38	87.25	15	20.19	4.77	47.28	4.13	66.32	2.38	0.9	0.6
T_3	90.88	86.52	15	20.85	5.00	45.67	4.36	66.32	2.38	0.9	0.6

*ME Mcal/kg

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

Sample Collection and Chemical Analysis: Feed offered were sampled and composited by animal for analysis. The samples of experimental diets and feces were dried at 55°C in a forced air oven and were ground to 2 mm particle size. These samples were analyzed for DM, CP, EE, CF and total ash by the methods of AOAC [16]. Chromic oxide was analyzed by atomic absorption spectrophotometer by the methods described by Williams, David and Iismaa [17]. Blood samples were collected by jugular veni-puncture monthly. Blood samples were centrifuged at 3000 rpm for 20 min and serum was harvested and stored at -20 °C until assayed for total proteins, albumin and total cholesterol.

Rumen liquor was taken from each calf in the last day of digestion trail for 3 periods at 0, 3 and 6 h post morning feeding by stomach tube. The rumen liquor was filtered through a double layer of cheese cloth into plastic tubes (50 ml). The filtrate used in determination of pH immediately after the sampling using a portable pH meter (Beckman, model 45, USA). Rumen liquor was used to determine total volatile fatty acids (TVFA's) concentration according to method as described by Warner [18], while rumen ammonia nitrogen (NH3-N) concentration was determined according to El-Shazly [19] and Abou-Akkada *et al.,* [20].

Analysis of Blood Metabolites: Blood metabolites were analyzed by spectrophotometer (Unico, USA) using commercial test kits (Spinreact, Spain).

Statistical Analysis: Statistical analysis was carried out using SPSS program, version 16.0 [21]. Differences between groups in nutrient digestibilties, rumen fermentation, blood metabolites and performance were evaluated by one way ANOVA. Differences among means were tested using Duncan's multiple range tests. The data were presented in mean ± SEM Level of significance was set at $P<0.05$.

RESULTS

Nutrient Digestion: The apparent digestibilties of DM, CP and CF in buffalo calves fed diets supplemented with caraway seed (CS) or dried garlic (DG) powder or both were 2.8, 3.93, 11.57, % higher ($p<0.05$) than those fed the control one, respectively. Also the EE was 4.37 % higher in garlic supplemented group compared to the control one (Table 3). Buffalo calves supplemented with both CS and DG exhibited the best nutrient digestibility results followed by garlic, then caraway supplemented group in comparison with the control group. However, OM and NFE digestibilties did not show any treatment ($p>0.05$) effect in buffalo calves fed experimental diets.

The % of digestible CP, CF, EE were significantly ($p<0.05$) higher in buffalo calves fed diet supplemented with CS or DG or both than those fed the control diet (Table 4). However, the % of digestible NFE was significantly higher in buffalo calves fed the control diet.

Table 3: Effect of dietary treatments on nutrient apparent digestibilties, %

	Control	T_1	T_2	T_3	P
DM	58.27±0.81^{b}	59.58±0.29^{ab}	60.64±0.43^{a}	61.13±0.63^{a}	0.007
OM	61.6±0.77	62.48±0.31	62.17±0.52	63.02±0.56	0.36
CP	69.55±0.83^{b}	71.97±0.23^{a}	69.88±0.84^{b}	73.48±0.61^{a}	0.001
CF	46.75±1.81^{b}	48.89±1.23^{b}	54.11±1.85^{a}	58.32±1.04^{a}	0.0001
EE	78.21±1.31^{b}	78.90±1.1^{b}	82.58±0.31^{a}	80.23±1.31^{ab}	0.04
NFE	63.60±1.51	63.05±0.90	60.99±1.18	59.92±0.47	0.07

Means within row bearing different superscripts differ significantly ($p< 0.05$).

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

Table 4: Effect of dietary treatments on percentage of digestible nutrient

Item	Control	T_1	T_2	T_3	P
CP, %	10.80±0.25^{c}	12.39±0.07^{a}	11.58±0.14^{b}	12.52±0.10^{a}	0.0001
CF, %	9.34±0.36^{c}	9.80±0.25^{c}	10.92±0.37^{b}	12.16±0.22^{a}	0.0001
EE, %	3.06±0.05^{c}	3.64±0.05^{b}	3.94±0.01^{a}	4.01±0.07^{a}	0.0001
NFE, %	35.85±0.85^{a}	33.27±0.48^{b}	33.14±0.64^{b}	31.61±0.25^{b}	0.0001
TDN, %	62.86±0.70^{b}	63.65±0.30^{bc}	64.51±0.45^{abc}	63.97±0.48^{a}	0.010
SE Theoretical	59.58±0.70^{b}	62.36±0.30^{bc}	63.22±0.45^{ac}	64.79±0.48^{a}	0.015
SE true	47.98±0.70	50.76±0.30	51.62±0.45	53.19±0.48	0.071

Means within row bearing different superscripts differ significantly ($p< 0.05$).

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

SE= starch equivalent

Table 5: Effect of dietary treatments on rumen fermentation parameters

	Control	T_1	T_2	T_3	p
pH					
0 h	7.15±0.13	7.47±0.47	7.49±0.18	7.46±0.06	0.76
3 h	6.09±0.11	6.14±0.54	6.41±0.25	5.6±0.14	0.38
6 h	5.57±0.18^{ab}	6.83±0.12^{a}	6.16±013^{b}	6.36±0.07^{b}	0.03
Total VFA's					
Mg Equiv./100 ml					
0 h	10.77±0.21	11.33±0.25	11.17±0.25	11.33±0.27	0.35
3 h	11.38±0.18^{ab}	13.44±0.52^{b}	12.16±0.46^{ab}	16.1±0.45^{a}	0.001
6 h	11.05±0.17^{c}	12.61±0.44^{b}	11.44±0.28^{bc}	16.12±0.73^{a}	0.001
NH_3-N mg /dl					
0 h	22.46±0.31	23.56±1.31	22.74±0.18	23.87±1.16	0.65
3 h	35.46±0.93^{a}	34.84±0.67^{a}	32.00±1.24^{a}	27.67±1.68^{b}	0.001
6h	27.07±0.52^{a}	19.60±0.66^{b}	19.91±0.72^{b}	19.28±0.56^{b}	0.001

Means within row bearing different superscripts differ significantly ($p< 0.05$).

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

NH_3-N =ammonia nitrogen VFA's=volatile fatty acids

Table 6: Effect of dietary treatments on blood metabolites

	Control	T_1	T_2	T_3	P
Total protein (g/dl)	6.63±0.14^{b}	7.28±0.16^{a}	7.64±0.04^{a}	7.76±0.24^{a}	0.001
Albumin (g/dl)	4.07±0.22	3.69±0.15	3.74±0.14	4.15±0.23	0.26
Globulin (g/ dl)	2.55±0.21^{b}	3.6±0.15^{a}	3.89±0.18^{a}	3.61±0.3	0.001
al/glo ratio	1.77±0.27^{a}	1.04±0.07^{b}	0.98±0.07^{b}	1.25±0.17^{b}	0.01
ALT (U/l)	51.78±2.15^{a}	44.22±1.87^{b}	43.22±1.06^{b}	42.22±1.47^{b}	0.001
AST (U/l)	58.44±30.2	20.11±1.36	17.77±1.13	16.33±0.94	0.169
Cholesterol mg/dl	242.99±7.4^{a}	198.78±9.3^{b}	155.5±4.4^{c}	161.8±7.2^{cd}	0.001

Means within row bearing different superscripts differ significantly ($p< 0.05$).

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

Table 7: Performance of buffalo calves fed experimental diets

Period in months	Control	T_1	T_2	T_3	P
Initial weight, kg	169.0±31.58	171.0±9.61	170.0±35.12	170.0±35.68	1.00
Final weight, kg	279.67±45.05	293.33±8.82	291.67±32.45	294.67±32.67	0.95
Daily gain, kg	o.614±0.1	0.679±0.01	0.675±0.04	0.692±0.01	0.77
Feed conversion	12.94±1.84	10.8±0.16	10.93±0.8	10.66±0.27	0.58
DMI kg/day	7.31±0.09	7.34±0.07	7.32±0.08	7.4±0.07	0.93
Daily feed cost/day E.P.*	9.33±0.1	9.32±0.17	9.46±0.04	9.39±0.22	0.63
Feed cost/kg WG** (E.P.)	15.92±2.34	13.7±0.28	14.14±1.02	13.57±0.36	0.57

Means within row bearing different superscripts differ significantly ($p< 0.05$).

T_1= Caraway seed (CS) T_2= Dried garlic (DG) T_3= CS +DG

* E.P. =Egyptian pound ** WG=Weight gain

Rumen Fermentation: No significant differences Among dietary treatment groups regarding the rumen pH at 0 and 3 hours after feeding (Table 5). However after 6 hrs there was a significant ($p<0.05$) increase in the rumen pH in the buffalo calves fed treated diet compared with those fed the control diet. In addition, rumen total volatile fatty acids were significantly ($p<0.05$) increased in the buffalo calves fed treated diet compared with those fed the control after 3 and 6 hours from feeding. Moreover, rumen ammonia nitrogen significantly ($p<0.05$) reduced in the buffalo calves fed treated diet compared with those fed the control diet after 3 and 6 hours from feeding.

Blood Metabolites: There were significant ($p<0.05$) increase in serum total protein and globulin in the buffalo calves fed treated diet compared with those fed the control. On the contrary, serum total cholesterol and ALT were significantly decreased ($p<0.05$) in the buffalo calves fed treated diet compared with those fed the control one (Table 6).

Calves Performance: Intake of DM was slightly increased ($p>0.05$) in buffalo calves fed diets containing both CS and DG than the control diet. The average daily weight gain (ADG) of calves fed experimental rations was 614, 679, 675 and 692 g, respectively (Table 7). Although the ADG remained unaltered across all treatments the calves fed ration supplemented with both garlic and caraway gained more than the other groups. Feed conversion efficiency (FCE) by buffalo calves didn't show any treatment ($p>0.05$) effect (Table 7). The feed conversion efficiency (FCE) was 12.99, 10.8, 10.93 and 10.66 in buffalo calves fed experimental rations, respectively (Table 7). Calves fed ration supplemented with both garlic and caraway gained maximum with least quantity of feed consumed per kilogram live weight gain.

DISCUSSION

Recently, there has been a renewed interest in using herbs as feed additives in ruminant nutrition. Among these products, garlic, caraway and essential oils are attracting much attention [22]. The significant increase in DM, CP, CF, EE digestibility was in agreement with results of El-Ashry *et al*. and Abo El-Nor *et al*. [23, 24]. In addition, some studies indicated that the medicinal plants improved rumen activity and nutrient digestibility [25-27]. Supplementation of garlic extract resulted in proper maintenance of liver function because it has an important protective role against liver toxicity caused by a variety of medicinal and environmental substances [28]. Further improvement in the digestibility coefficients of different nutrients is probably due to garlic which enhances the activity of pancreatic lipase and amylase [29] and modify the numbers and species of microorganisms in the rumen [30].

The improvement in rumen fermentation parameters in terms of increased total VFA`s was in agreement with results of [5, 31, 32, 34]. Kongmun *et al.* [33] suggests that supplementation of garlic could improve ruminal fluid fermentation in terms of volatile fatty acid profile, reduced methane losses and reduced protozoal population. In this respect The reduction in rumen ammonia was similar with results of Cardozo *et al.* [34] ; Benchaar *et al.* [35] and Kongmun *et al.* [33] who reported that the addition of garlic powder or oil *in vitro* reduced ammonia N, suggesting that the deamination was inhibited.

The improvement in serum total protein, globulin and albumen/globulin ration are agreement with those reported by El-Ashry *et al*. [23] and Abo El-Nor *et al.* [24] and Ahmed *et al.* [36] who found that the mean values of blood metabolites were higher in treated animals with medicinal herbs than control.

The lower serum total cholesterol in treated groups could be ascribed to garlic which is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol [37], inhibit bacterial growth [38], inhibit platelet growth and reduce oxidative stress [39].

The numerical improved BW gain and feed intake, feed conversion of treated group was consistent with results of Ahmed *et al.* and Ghosh *et al*. [36, 40]. The little improvement In performance in the present study may result from improved enteric health due to the chemical constituents of garlic and caraway which had antioxidant, antibacterial and antifungal activities and also stimulate and metabolism [40]. It is possible that diets containing garlic exert a positive effect on the balance of gut microbial populations [41] which may be responsible for this positive result. Ahmed *et al*. [36] reported that using garlic, anion and lemonade juice as natural feed additive at the rate 2.5, 5% and 7.5%/kg ration for growing buffalo calves improved utilization of nutrients, better blood constituents, improved growth rate, destroy harmful bacteria and cause some improvement of economical efficiency. The group fed supplemented diet with 2.5% natural additive showed the best performance. On the other hand, some studies confirmed such plants seeds had favorable effects on nutrient digestibility, live weight and feed efficiency with cows [26, 27, 40, 42].

CONCLUSION

Caraway and garlic can be supplemented to growing buffalo calves for better nutrient digestibilties, performance, rumen fermentation, metabolic status and general health status.

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