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Adding Natural Juice of Vegetables and Fruitage to Ruminant Diets (B) Nutrients Utilization, Microbial Safety and Immunity, Effect of Diets Suplemented with Lemon, Onion and Garlic Juice Fed to Growing Buffalo Calves

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Abstract: Twenty four buffalo calves weighted 120.49±0.7 Kg and 4.5±0.3 month of age were selected and divided randomly into 4 similar groups (6 animals each) in 140 days feeding trial. All calves in the different groups were fed similar basal diet of concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS). First group (D_1, control) fed the basal diet. Group 2, 3 and 4 (D_2, D_3 and D_4) were supplemented with 2.5, 5 and 7.5 % /kg diet/day natural additive of juice of garlic, anion and lemonade (1: 1: 0.125 / liter clean water) respectively. Bacteriological tests were done in all diets. At the end of 8th week of the feeding trial, the digestibility traits were carried out using twelve buffalo calves (3 calves each) to evaluate the pervious rations by using acid insoluble ash (AIA) technique. Samples of blood were drowning to evaluate hemaotcrate (Ht) count, red blood cells (RBC) count, white cells (WBC) count and its components and immunity. Results indicated that digestibility of all nutrients as well as DCP values were significantly increased ($P<0.05$) by supplementing diet of growing calves with 2.5% natural additive (G2) which showed the best diet.. Values of TDN did not significantly affected by any level of supplement, G2 tended to be the best. Intake in terms of DM, TDN or DCP did not significantly by natural additive. Blood components GPT and GOT were significantly increased ($P<0.05$) by increasing natural additive. Similarly, red blood cells and white blood cells increased ($P<0.05$). Immunity (globulins fractions) increased in treatments group than that control group. Meanwhile, total lipids declined by increasing level of natural additives. Average daily gain was increased by 4.8% in animals of D_2 but the daily gain was significantly ($P<0.05$) decreased with the higher levels of natural additive 5 and 7.5 % (D3 and D4) comparing with D2 and insignificantly comparing with the control animals. For the microbiological quality of used feeds (FCM, BH and RS), the increasing level of supplementation decreased TPC, TCC and FCC.Similarly, enterobacteria, *E.coli* and *Salmonella* not detected. Also, pH values were significantly ($P<0.05$) decreased by adding natural additive. It could be concluded 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.

Key words: Buffalo calves · Lemon onion and garlic juice · Growth performance digestibility · Immunity and microbiological quality of the diet

INTRODUCTION

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 in addition to be used as rumen manipulators to increase animal productivity. Attempt to use natural materials such as medicinal plants could be widely

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accepted as feed additives [1,2]. Using medicinal herbs and plants with humans has been known since the old civilizations. Old drugs industry depended upon the raw material of medicinal herbs and plants and their extracts, which proved safe. Inversely many synthesized chemicals additives caused many hazards to animal, plants and humans. The world health organization (WHO) encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature [3].

Garlic, onion and lemonade juice as feed additive is experiencing a resurgence in animal health and production too. However, the risk of the presence of antibiotic, residues in milk and meat and its effects on human health have led to its prohibition for use in animal feeds in the European Union in 2006 [4]. Because some of the vegetable and fruits entail oils and citric acid that highly inhibitory to some pathogenic and spoilage microorganisms, this may provide alternatives and supplements to conventional antimicrobial additives in foods. Many investigators reported that garlic and onion are highly inhibitory to *E.coli* and to other bacteria and fungi.,e.g antibacterial and antifungal [5-7], as antifungal [5, 7], as enzyme inhibitory [7]. Activities of garlic and onion have been widely studied, the active inhibitory principle of garlic is allicin or daily thiosolphinic acid [8]. Allicin is enzymatically released from precursor form when the garlic and onion bulbs are crushed.

Several reports suggested that consumption of dietary antioxidants (vegetables and fruits) or supplementation of antioxidants could have a protective role against degenerative diseases of aging [9, 10]. Garlic and onion biological action products are ascribed to its sulfur-containing active principle, mainly in the form cysteine derivatives. Garlic and onion have been reported to effectively prevent lipid levels in experimental animal [7]. In addition to their lipid lowering effects, garlic and onion reparations have been shown to inhibit oxidation of low-density lipoproteins. Wangensteen *et al.* [11] showed that addition of natural additives onion and garlic to food will increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes.On the other hand, Gupta *et al.* [12] and Aid *et al*. [13] found that improvement in the digestibility coefficients of different nutrients is probably due to improved gross activity of rumen microflora, increased immunity alternation in numbers and species of microorganisms in the rumen on inclusion vegetable an id fruits increase in cellulolytic bacteria, increased total volatile fatty acids (TVFA·s) concentration and the animals rations and higher DM, TDN intake and more higher gain rate.

The objective of the present study was to evaluate of natural additive (vegetables and fruits), garlic, onion and lemonade juice as feed additives on feed intake, nutrient digestibility, growth performance, immunity and economical efficiency of growing buffalo calves and as an inhibitory for diet harmful microorganisms.

MATERIALS AND METHODS

This study was carried out at Mahallet Moussa Experimental Station and By-Product Utilization Department, Animal Production Research Institute (APRI) and Regional Center for Food and Feed Agriculture Research center (RCFF), Ministry of Agriculture, to evaluate the effect of supplementation diets with natural additive on growth performance immunity and some blood traits of growing buffalo calves. In this study twenty four growing buffalo calves with an average of live body weight 120.49±0.7Kg and 4.5±0.3 month of age were used. The experimental calves were divided randomly into four groups (6 each) Calves of the 1st group (as control) reared on CFM (26%, undecortecated cotton seed meal, 44% wheat bran, 19% yellow corn, 5% rice bran, 1% salt mixture, 2% lime stone and 3% molasses), berseem hay (BH) and rice straw (RS) without additive. Calves of the 2nd, 3rd and 4th groups received the same ration with supplemented of mixture of juice of garlic, onion and lemon in a molar 2.5, 5 and 7.5% /kg diet /day for treatment groups respectively. The juice was prepared and sprayed as Aiad *et al* [13]. Representative composite samples of the experimental diets and feed ingredients were taken for chemical analysis, the data are presented in Table 1.Calves were housed in three open shaded paddocks and daily offered the experimental rations as 2 % CFM + 0.5% berseem hay (BH) of their body weight. Feeds were offered in two equal portions at 8.00 a.m. and 4.00 p.m. as described by APRI [14] which cover their nutritional requirements as NRC [15] recommendations. Rice straw as a bulky roughage was offered *ad lib*. Fresh water and mineral mixture blocks were freely available to animals. The growth trial lasted 140 days. Calves were biweekly weighed in the morning before offering any feed or water. Live body weight changes and feed intakes were recorded at biweekly intervals.

Table 1: Chemical composition of the feed ingredients and experimental diets (on DM basis)

Items	D1	D2	D3	D4	CFM	RS	BH
Moisture	8. 86	8. 64	8. 55	7.75	8.45	7.85	9.71
DM composition (%)							
OM	91.14	91.51	91.45	91.25	87.11	81.50	87.50
CP	14.32	14.26	14.26	14.04	17.53	3.92	12.30
CF	16.82	17.32	17.70	16.82	7.95	35.24	28.50
EE	3. 51	3.53	4. 00	4. 60	2.46	0.46	2.70
NFE	56.49	56.40	55.49	55.79	59.17	41.88	44.00
Ash	8.86	8.49	8.55	8.75	12.89	18.50	12.05
DE kcal/kg*	2.40	2.39	2.37	2.40	2.69	1.81	2.02

*DE=4. 36 – 0. 049 x NDF

NDF = 28. 924 + 0. 657 (CF %) according to Cheeke [36]

CFM: 26%, undecortecated cotton seed meal, 44% wheat bran, 19% yellow corn, 5% rice bran, 1% salt mixture, 2% lime stone and 3% molasses.

Digestibility Trails: At the middle of the growth experiment, three calves were taken at random from each group to determine the digestibility and nutritive value of experimental rations. Acid insoluble ash (AIA) method was used as described by Van keulen and Young [16]. During feces grabbing period calves were fed 2 % CFM, 0.5% BH and chopped rice straw as 80 % from the average daily free choice intake. Feed and rectum grabbed feces collection was practiced for 5 days. Feces samples were treated with 10 % sulfuric acid (H_2SO_4) and kept frozen at -18°C for further chemical analysis. The chemical analysis of feeds and feces were carried out according to AOAC [17].

Blood Samples: Blood samples were collected biweekly from the jugular vein during experimental period. Few drops of whole blood were drawn into heparin-coated tube at each sampling time for determinant of hematocrate (Ht), hemoglobin (Hb), red (RBCs) and white blood cell count (WBCs) and the different types of (WBCs) were also counted. The test samples of the blood were centrifuged at 4000 rpm for 20 minutes. The obtained serum was stored at -20°C until analyzed. Determination of levels of immunoglobulin IgG, IgM and IgA was done by bovine radial immune diffusion sit (RID) kit according to technique procedure outlined by the manufacture (The binding site Ltd. Birmingham, UK). The principle of the technique was derived from the work of Mancini *et al*. [18] and Fahey and Mekelvey [19]. The serum of total protein and albumin were determined according to Doumas *et al*. [20] and globulin was estimated by difference. The activity of SGOT and SGPT were determined according to Reitman and Frankel [21]. Total lipids were determined according to Rattief and Hall [22].

Dietary Microbiological Evaluation: Appropriate dilutions prepared from each sample were used for inoculating different nutrient and selective media. The microbial determinations were applied as follows:

Total Aerobic Viable Counts: Aerobic bacterial counts were estimated on glucose yeast extract nutrient agar medium as the method reported by A.P.H.A. [23] using pouring plate technique. Suitable plates were counted after incubation at 37°C for 48 hours.

Coliform and Feacal Coliform: Coliform and feacal coliform counts were estimated on Macconkey agar [23] using pouring plate technique. Suitable plates were counted after 24 hours at 37°C and 44.5°C for total coliform and feacal coliform counts, respectively.

Enterobacteriacea Counts: Violet red bile glucose agar medium plates [24] were incubated with 1 ml of the appropriate dilutions and incubated overnight at 37°C. After incubatation on clearly visible purple colonies surrounded by a purple halo were estimated as enterobacteriaceae counts [25].

Detection of *Salmonella:* The methods of Georgala and Boothroyd [26] and Khan and McCaskey [27] was applied by adding 225ml peptone water as pre enrichment medium to twenty-five g. of each sample and incubated at 37°C for 24 hours. After incubation the culture was streaked on difco brilliant green agar plates and examined after 25-28 hours (on this medium presumptive salmonella appears as pink colonies surrounded by bright red medium).

Enumeration of *Escherichia coli* 0157:H7: Culture media and imurogenetic separation reagents, the enrichment medium was modified tryptone soya broth (mTSB=N) containing novobiocin solution 20 mg/liter of (mTSB) [28, 29] and the subculture on medium sorbitol macconkey agar [25] containing defixime 1ml /liter and potassium telluride 1ml/ liter of sorbitol Macconkey agar (Cefixime etllurite sorbitol Macconkey agar (CT-SMAC)[30].

pH Values: The value of pH was measured according to Ling [31]. pH values of samples were estimated by means of an electric pH-meter (Wissenscha thich-technich werkstatten D8120 Weilheim pH40).

Statistical Analyses: The data were statistically analyzed using GLM produces of SAS [32]. Duncan's test [33] was applied in experiment whenever to test differences.

The following model was used:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where:
Y_{ij} = observed trait, μ = overall mean,
Ti = effect of treatment e_{ij} = random error

RESULTS AND DISCUSSION

Chemical Composition: The chemical composition of feed ingredients and experimental diets are presented in Table 1.Data indicated that the experimental diets were isonitrogenous and isocaloric. The values of CP for the experimental rations ranged between 14.04 to 14.32. The chemical composition of the BH and RS were within the normal published ranges for CP, CF and DE [34]. The chemical compositions of CFM were nearly similar reported by Aiad [35]. The composed diets showed comparable nutrients.

Nutrients Digestibility and Nutritive Values: Data in Table 2 indicated that the supplemented diet with 2.5% natural juice (D2) fed to the growing calves significantly ($P<0.05$) tended to improve the digestibility coefficients of all nutrients and showed the highest values. These findings reflected that the level 2.5% may be is the most suitable concentration for rumen activity compared with other ratios in D3 and D4, but the improvement in OMD, CFD and EED was not significant compared with the control diet (D1). On the other hand, the diet (D4) which supplemented with 7.5% of the natural juice showed significantly ($P<0.05$) the lowest values of digestibility

Table 2: Dry matter intake, nutrient digestibility and nutritive values of different experimental diets fed to buffalo growing calves

	Experimental diets				
Item	D1	D2	D3	D4	±SE
Animal weight, kg	159.83	164.40	158.74	160.99	±4.90
DMI intake (kg /head /day)					
CFM	3.596	3.699	3.572	3.568	±2.48
BH	1.598	1.644	1.587	1.633	±1.39
RS	0.479	0.493	0.476	0.482	±2.42
Total DMI kg/head/day	5.673	5.836	5.635	5.683	±1.26
Nutrient digestibility %					
DM	69.72 [b]	71.43 [a]	69.11 [b]	66.85 [c]	±0.61
OM	71.16 [a]	72.25 [a]	71.07 [a]	68.37 [b]	±0.68
CP	66.47 [a]	66.63 [a]	62.23 [b]	63.22 [b]	±0.39
CF	61.73 [b]	65.66 [a]	61.92 [b]	62.04 [b]	±1.42
EE	80.90[a]	82.81 [a]	81.14[a]	75.16 [b]	±1.26
NFE	75.39 [a]	75.59 [a]	75.32 [a]	74.06 [b]	±1.21
Nutritive value %					
TDN	67.71	67.89	66.84	67.31	± 0.25
DCP	9.52 [a]	9.50 [a]	8.87[b]	8.88 [b]	±0.22
TDN intake					
Kg /head / day	3.84	3.93	3.77	3.85	±0.03
Kg /100 kg body weight	2.29	2.29	2.25	2.28	±0.30
g / kg $W^{0.75}$	81.46	82.22	79.95	81.21	±1.48
DCP intake					
Kg /head / day	0.540	0.554	0.498	0.531	±2.03
Kg /100 kg body weight	0.3380	0.3373	0.3138	0.3300	±0.42
g / kg $W^{0.75}$	12.02	12.08	11.14	11.75	±0.05

[a, b, c] Means at the same row having different superscripts are significantly different at ($p< 0.05$)

Table 3: Some blood parameters of growing buffaloe calves fed on supplemented diets with natural additive

	Experimental groups				
Item	D1	D2	D3	D4	±SE
Total protein g/dl	5.83	6.02	6.10	6.00	±0.14
Albumin g/dl	3.23 [c]	3.34 [b]	3.36 [b]	3.62 [a]	±0.11
Globulin g/dl	2.60 [b]	2.38 [b]	2.74 [a]	2.48 [b]	±0.08
Total lipids g/dl	6.19 [a]	6.04 [a]	5.03 [b]	5.01 [b]	±0.09
GPT	21.35 [c]	23.84 [a]	24.68 [a]	22.75 [b]	±0.96
GOT	63.75 [c]	65.63 [b]	68.02 [a]	63.58 [c]	±1.35

[a, b, c] Means at the same row having different superscripts are significantly different at ($p< 0.05$).

which indicated that level may be inhibit the rumen activity. Regarding the level 5% of supplementation, the values of digestibility of all nutrients did not significantly ($P<0.05$) affected compared with the control diet except for CFD it was lower. So, that level may inhibit the activity of cellulolytic microorganisms. These results are in agreement with those found by El-Ashry *et al*. [3], Aiad *et al*. [13], Moawad [37], Zaki *et al*. [38] and Khir and Ibrahim [39].

Regarding feeding values, presumably trends and values of nutrient among treatments ultimately on its DM. Accordingly, insignificant differences ($P<0.05$) were observed among all diets fed to the growing calves for TDN values. While, values of DCP showed insignificant differences between D1 and D2 and between D3 and D4.The first groups (D1 and D2) were significantly higher ($P<0.05$). Daily feed intake of growing calves per metabolic body weight as TDN and DCP seemed to be the highest with 2.5% natural juice supplemented diet (D2), but, the differences were insignificant.These findings means that the best level of supplementation was 2.5%. These results are in harmony with those reported by El-Ashry *et al*. [3], Aiad *et al*. [13], Zaki *et al*. [38] and El-Ashry *et al*. [40].

Blood Biochemical Parameters: Data in Table 3 showed that serum total protein were not significantly by the natural additive. Bush [41] reported a positive correlation between dietary protein and plasma protein concentration. This indicates that the supplement had not affect protein synthesis in liver function. The highest values of serum albumin were significantly ($P<0.05$) observed with G4 which fed supplemented diets with 7.5% natural additive. These results agreed with those found by El-Ashry *et al*. [3]. Also, Bush [41] reported that the low level of proteins may be attributed to a decrease in the protein absorbed and synthesized and an increase in protein losses. The highest values of serum globulin, GOT and GPT (glutamic-pyruvic and glutamic-oxaloacetic transaminases) were observed for animals of D3 which fed the supplemented diet with 5% natural additive. The lowest values total protein, albumin, globulin, GOT and GPT were recorded for the control group (D1), but it recorded the highest value just for total lipids which decreased with increasing level of the natural additive.

The present results agree with those of Lau *et al*. [42], who found that additive garlic lowered total lipids and high values of the blood components. The prepent results are also in harmony with the findings of Prasad *et al*. [43] and El-Hosseiny *et al*. [44], who mentioned that additive garlic oil lowered serum lipids and higher blood components in small ruminant.

Heamatological Response and Immunoglobulins: Average red blood cells (RBC's), white blood cells (WBC's) counts and hematocrit (Ht) concentration are illustrated in Table 4. It clear that natural additive supplementation had significant ($P<0.05$) on RBCs and Ht. Their values increased when the additive increased. These results agreed with those reported by El-Gafrawy *et al*. [45], they did not find any effect of additive in early calves' age. But, increased significantly at later age. Table 4 indicated significant effect ($P<0.05$) of additive supplementation on the percentage of lymphocyte, monocyte, basophile and eosenophile. The percentage of white cells content tended to increase with the increased of juice level. El-Gaafrawy *et al*. [45] and Beanarek *et al*. [46] found that calves aged 3-5 weeks received the additive selenium and alphatocopherla acetate, showed blood white cells content and greater phagocytosis than the control. In general, natural additive content (onion and garlic) their components TVFA's and essential amino acids reflected to digestion in the rumen and early destroy any infection in growing calves.

Table 4: Hematological response and immunoglobulin fractions of growing buffaloe calves fed supplemented diets with natural additive

	Experimental groups				
Item	D1	D2	D3	D4	±SE
Red blood cells					
Ht	12.26 [b]	13.23 [a]	14.93 [a]	13.39 [a]	±0.17
RBCs	10.82 [c]	10.10 [c]	12.75 [a]	11.43 [b]	±0.15
White cells(%)					
Lymphocyte	45.36 [c]	49.98 [b]	54.95 [a]	51.59 [b]	±0.60
Munocyte	3.44 [b]	3.95 [a]	4.12 [a]	3.98 [a]	±0.03
Basophil	0.20 [b]	0.21 [b]	0.28 [a]	0.3 [a]	±0.02
Esenophil	3.18 [b]	3.67 [a]	3.63 [a]	3.99 [a]	±0.11
Immunoglobulin fractions					
IgG	46.24 [c]	50.15 [b]	53.15 [a]	47.24 [b]	±1.10
IgM	2.91 [c]	3.27 [a]	3.38 [a]	3.11 [b]	±0.12
IgA	2.27 [c]	2.85 [a]	2.92 [a]	2.70 [b]	±0.19

[a, b, c] Means at the same row having different superscripts are significantly different at, ($p< 0.05$).

Immunoglobulin Fractions (IgG, IgM and IgA): Table 4 showed that the concentration of immunoglobulins (IgG, IgM and IgA) in serum samples increased significantly ($P<0.05$) in both D_2 and D_3. The lowest values were observed for the control group D1. This displays the fact that immunoglobulin increased markedly as a result of ingestion of complete ration. The present results are comparable to those reported by El-Ashry *et al*. [3], El- Gaafrawy *et al*. [45], Ishikawa and Konishi [47] andrews *et al*. [48], Ikeuchi *et al*. [49] and Tizard [50]. Starting from the 3rd week of age, there was significant ($P<0.05$) increase in serum IgG concentration in treated group than those of control. Whereas, the significant ($P<0.05$) increase in serum IgA concentration in the treated group started at the 7th week. Serum of treated groups significantly ($P<0.05$) increased IgM concentration compared with the control group. Serum IgA was higher in D_2 and D_3 than D_1 and D_4. These results may be related to the role of the antioxidant nutrients (natural additive) which can modulate and regulate the early activation steps in the acquired immune response. El-Gaafrawy *et al*. [45] and Bednarek *et al*. [46] found that intramuscular injection of calves with 5.75 mg selenium and 75 mg alpha-tocopherol acetate caused greater gamma-globulin than that of control. The aforementioned increase of immunoglobulin (IgG, IgM and IgA) could be credited to B-lyphocyte stimulation.Consequently the immunoglobulins will elevate initiating and immune response through helper T-cell, cytotoxic T. cells and C_8 –T- cell [30]. This may lead to a perfect immunocompetant calves. It should be recorded that no incidence of mortality cases, diarrhea or respiratory disease were noticed in calves received supplementation of natural additive. On the other hand, low cases of diarrhea and one case of respiratory diseases were observed throughout the experimental period in control group.

Growth Performance: Results in Table 5 indicated that natural juice of lemon, onion and garlic supplementation with different levels did not significantly affect the feed consumption as DM, TDN or DCP of buffaloe growing calves along the feeding trial compared with the control but G2 which fed on diet of 2.5% natural additive tended to be the highest. The results are in agreement with those reported by El-Ashry *et al*. [3], Aiad *et al* [35], Zaki *et al*. [38] and El-Ashry *et al*. [40]. The data of the daily gain of the growing calves revealed that feed additive of 2.5% natural juice (D2) significantly ($P<0.05$) increased compared with the control group (D1), showed the best level of supplementation compared with other levels 5 and 7.5% (D3 and D4) respectively. Also, (D2) showed the best feed conversion but the differences among all groups were not significant. These results might be due to the effective to improve immunity and decrease debility incidence, which agree with the findings of Aboul-Fotouh *et al*.[2], Safaa [51]and Aboul-Fotouh *et al*. [52], they reported that nutrition plays important role in diminishing growth rate.

Table 5: Growth parameters of growing buffaloe calves fed on supplemented diets with natural additive

	Experimental groups				
Item D1	D2	D3	D4	±SE	
Duration period / (days)	140	140	140	140	
Number of growing calves	6	6	6	6	
Average body weight (kg):					
Initial body weight (kg)	116.66	116.66	115.83	116.38	±2.11
Final body weight (kg)	253.80 [b]	260.30[a]	252..80[b]	255.63 [b]	±3.20
Total weight gain (kg)	137.14 [b]	143.64[a]	136.97 [b]	139.25 [b]	±0.29
Av. Daily gain (kg)	0.979 [b]	0.1.026 [a]	0.978 [b]	0.995 [b]	±0.15
DM intake (kg /head /day)					
CFM	3.463	3.491	3.370	3.441	±0.28
Berseem hay	1.539	1.547	1.495	1.527	±0.38
Rice straw	0.792	0.798	0.769	0.786	±0.18
Av. Total intake (kg /head /day)					
DMI 5.79	5.84	5.63	5.76	±0.83	
TDNI	3.92	3.93	3.77	3.87	±0.32
DCPI	0.55	0.55	0.49	0.51	±0.13
Feed conversion:					
Kg DMI/kg gain	5.92	5.67	5.76	5.79	±1.11
Kg TDN/kg gain	4.00	3.82	3.85	3.89	±1.11
Kg DCP/kg gain	0.56	0.54	0.51	0.51	±0.23

[a, b] Means at the same row having different superscripts are significantly different at ($p< 0.05$).

Table 6: Aerobic plate total, coliform, fecal coliform, enterobacteria, *Salmonella* spp and *E. coli* 01507 counts and pH values in examined hay, water and CFM without and before adding juice

Sample	Addition juice	TPC	TCC	FCC	Entr.	*Salmonella*	*E.Coli* 01507	Final pH
HAY	Without addition	$76x10^{-4}$	$45x10^{-4}$	$21x10^{-4}$	$66x10^{-3}$	N.D	+	7.02
	2.5%	$57x10^{-4}$	$38x10^{-4}$	$16x10^{-2}$	$40x10^{-3}$	N.D	N.D	4.44
	5%	$58x10^{-4}$	$34x10^{-3}$	$4x10^{-2}$	$35x10^{-3}$	N.D	N.D	3.96
	7.5%	$17x10^{-3}$	$25x10^{-2}$	$15x10^{-1}$	$30x10^{-2}$	N.D	N.D	3.83
Water	Without addition	$190x10^{-2}$	$60x10^{-2}$	$36x10^{-4}$	$77x10^{-2}$	N.D	+	3.33
	2.5%	$65x10^{-2}$	$40x10^{-2}$	$15x10^{-2}$	$50x10^{-2}$	N.D	N.D	3.19
	5%	$45x10^{-2}$	$9x10^{-2}$	$7x10^{-2}$	$30x10^{-2}$	N.D	N.D	3.5
	7.5%	$22x10^{-1}$	$16x10^{-1}$	N.D	$10x10^{-1}$	N.D	N.D	
CFM	Without addition	$85x10^{-2}$	$55x10^{-2}$	$45x10^{-2}$	$48x10^{-2}$	N.D	+	5.12
	2.5%	$68x10^{-2}$	$38x10^{-2}$	$25x10^{-2}$	$40x10^{-2}$	N.D	N.D	4.52
	5%	$30x10^{-2}$	$20x10^{-2}$	$10x10^{-1}$	$25x10^{-2}$	N.D	N.D	4.20
	7.5%	$17x10^{-2}$	$19x10^{-1}$	$3x10^{-1}$	$16x10^{-2}$	N.D	N.D	4.18

TPC: Total aerobic plate counts TCC: Total coliform counts FCC: Fecal coliform counts

Microbiological Quality of Supplemented Diets: Table 6 shows the mean of aerobic plate count, total coliform, fecal coliform and entero bacteria in diets with or without the natural additive. The mean aerobic plate count of hay, water and CFM were ($76x10^{-4}$, $45x10^{-4}$, $21x10^{-4}$ and $66x10^{-3}$), ($90x10^{-2}$, $320x10^{-2}$, $36x10^{-2}$ and $77x10^{-2}$) and ($85x10^{-2}$, $55x10^{-2}$, $45x10^{-2}$ and $48x10^{-2}$) respectively before addition of the natural additive. Total aerobic counts were not higher than the recommended safety. The mean total aerobic count for all nutrient feeds and water analyzed $7.3x10^{-3}$ (cfu/g). For instance, all samples having total aerobic counts (TPC) less than the recommended safety limit of 10^{-4} cfu/g proposed by the International Dietetics of Association of European Community (IDAEC) and the Egyptian standards. These findings are consistent with the quality of nutrient components and water examined in calves feeds, where the mean total aerobic counts obtained in various concentrations level were less than that without additive. The highest concentration level was the least than other concentration levels. The growth of the bacterial strains in different supplemented diets was less or not detected after the inhibition effect of the juice at various concentrations. The three levels of juice inhibited the bacterial growth and *Salmonella* spp. and *E. coli* 01507. Natural additive contains garlic, onion and lemon juice, garlic contains 0.3-0.5 allicin and antimicrobial

Table 7: Feed efficiency and economic efficiency of growing calves fed on experimental diets

	Experimental groups			
Item	D1	D2	D3	D4
Kg gain/kg DMI	0.17	0.18	0.17	0.17
Kg gain/kg TDNI	0.25	0.26	0.26	0.26
Kg gain/kg DCPI	1.78	1.87	1.99	1.95
Av. Daily gain (kg)	0.979	1.026	0.978	0.995
Daily feed cost (LE)	6.92	7.01	6.82	6.97
Price of daily gain(LE)	14.19	14.93	14.18	14.43
Economic efficiency (%)	2.051	2.130	2.079	2.070
Relative economical efficiency (%)	100	103.85	101.37	100.93

* Economical efficiency % = Price of daily gain/daily feed cost x 100. *price of CFM: 1.40 LE/kg.

*Price of BH: 0.80 LE/kg. *Price of RS: 2.00 LE/kg. *Price of mixed juice 0.20 LE/kg.

*Price of gain 14.5 LE/kg.

component [53]. According to Kumar and Berwal [7] and Zaika and Kissinger [54] the gram-positive are generally more sensitive to allicin than gram-negative bacteria. Acetic acid bacteria are the most resistant among the gram positive bacteria. Abdou *et al*. [55] concluded that 5-10 % fresh garlic was sufficient to inhibit the growth of *E.coli.* The same trend was observed in onion. Which all tested bacteria were sensitive to onion extract and citric acid.

In the present study pH reduction and bacterial counts were determined. CFM, hay and water, citric acid was produced and the pH dropped to a value 4.0, so antimicrobial products (bacteriocins) could not be detected. The results showed that the produced citric and acetic acid and the pH reduction in the calves feed are responsible for *Salmonella spp* inhibition in calves water and ration. Natural juice did not show any other antimicrobial effect on *Salmonella* and *E. coli*. The present results agree with Gherbawy [56].

Feed Efficiency and Economic Efficiency: Data in Table 7 showed that there were differences between animals fed supplemented diets and those fed control diet in feed efficiency calculated as gain related to intake of DCP. Growing calves fed control diet (D1) obtained the least feed efficiency compared with those fed supplemented diets in D2, D3 and D4. The most efficient one was group (D3) which fed the supplemented diet with 5% natural juice (lemon, onion and garlic). While the highest relative economic efficiency was recorded for calves fed supplemented diet with 2.5% natural additive compared with those fed the control diet (D1) by 3.55%. Approximately similar results were observed for (D3 and D4). These results are in agreement with those obtained by Aboul-Fotouh *et al*. [2],Aiad *et al*. [13] and Aboul-Fotouh *et al*. [52].

This study it be concluded that natural additives effect to improve average daily gain and increases dry matter intake will vary depending on its level, the ingredients fed high or low protein and fiber and also the animal response.

Generally, recommendation is that the optimal additive level based on animal health, nutrient digestibility and daily gain was 2.5%. Thereby, natural additive of juice of lemon, onion and garlic could be used successfully and safety in rations to improve the performance of growing calves.

Further studies are required to investigate the effect of the natural additive on

- Rumen turnover rate and microbial protein synthesis.
- Number of cellulolytic bacteria and protozoa.
- Active substances which improve animal performance and immunity and the diet quality.

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