RESPONSE OF SUMMER STRESSED GROWING RABBITS TO SOME DIETARY GROWTH PROMOTERS

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ABSTRACT

An experiment was carried out to investigate the response to some growth promoters as safe alternatives to antibiotics on some performance aspects of rabbits subjected to environmental stress of summer season (29–33°C) and 48–63% relative humidity. Seventy, 6 weeks old-NZW rabbits were equally sexed and allotted to evaluate one of the following seven feed additives: 1: no supplement, 2: 0.5% acetic acid, 3: 0.5% lactic acid, 4: 0.5% a herb mixture of equal parts of sage+oregano+sweet basal, 5: 1.0% of the previous herb mixture, 6: 1.0 g GOS® (gluco-oligosaccharides; a prebiotic)/ kg diet, and 7: 1.0 g Bio-Mos® (mannan-oligosaccharides; a prebiotic)/ kg diet. The growth trial lasted up to the 13th week of age. Results reveal that total weight gain (p<0.01), and feed conversion ratio (p<0.05) were improved, while a significant decreasing effect (p<0.05) in both plasma total lipids and cholesterol with supplements under studied was detected. 0.5% acetic acid, or 1.0% herb mixture decreased (p<0.01) the abdominal fat content of the carcass. Histological study indicated that villi height and crypt depth, both (p<0.01) were increased with studied additives. pH of blood, cecum and ileum, besides, cecal content of volatile fatty acids, all were not affected by supplements. Conclusively, it is advisable to fortify rabbit diets reared under elevated ambient temperatures with one of the suggested additives, especially, with the prebiotics GOS®, Bio-Mos® or the herb mixture at 0.5% supplementation level to improve growth and health of rabbits grown under high ambient temperature conditions.

Keywords: rabbit, summer season, performance, organic acids, medicinal plants, and prebiotics

INTRODUCTION

High ambient temperature coincided with elevated relative humidity and digestive disorders around weaning are the most limiting factors in rabbit production in the tropics and subtropics. Therefore, feed additives which enhance performance under these critical conditions are of great interest for nutritionists and stockman (Abdel-Khalek., 2003 and Fonseca *et al.,* 2004). Recent evidence on the development of resistance in zoonotic organisms of animal origin and consumers' claims for safety animal foods urged the necessity for other natural, safe, reliable, and economic additives that serve the same goals achieved by antibiotics.

Organic acids, besides its anti-biotic like action, through inhibiting action for the intestinal bacteria competing with the host for available nutrients (Hyden, 2000), serve as substrates in the intermediary metabolites, increase gastric proteolysis and availability of some elements that complex with (Kirchgessner and Roth, 1988). Upon rabbit weaning, a predisposing factor to diarrhea is reduced acidity (a more alkaline pH) of the cecal contents, administrating a source of acid increased the cecum acidity and may reduce enteritis incidence (Morisse *et al.,* 1985). It seems that when evaluating the effect of organic on rabbit performance, hardly any trends could be demonstrated. On one hand, El-Kerdawy (1996) and Scapinello *et al.,* (1998) reported encouraging results when evaluating different sources of organic acids in rabbit diets; on the other hand, El-Allawy (2001) reported no advantage to supplement organic acids to rabbit.

Essential oils from herbs have anti-microbial and anti-oxidant activities also have hypo-cholesterolemic effects, indeed, a growth enhancing effect has been reported (Lee *et. al*, 2004). Sag extracts contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids (Ho *et al.,* 2000). Terpenoids in the dried green leaves in sage (*Salvia officinalis L.*) were regarded as the most promising sources of natural anti-oxidants, where sage protected against H2O2-induced DNA damage (Aheren *et al.,* 2007) and play an important role in protecting mitochondria function against various oxidative stress (cited from Ibrahim *et al.,* 2002). When incorporated at a range of 0.25–1.0% in growing rabbit diets, increased live weight gains, improved feed conversion ratio, but fluctuated plasma values of total protein, total lipids and cholesterol were reported (Ibrahim *et al.,* 2002 and Khayyal, 2006). The main components in oregano-oil (*Origanum vulgare L.*), are carvacrol (60–80%) and thymol (2%); carvacrol inhibits the metabolism of the microbial cell wall (Smink, 2000). Indeed, has a significant antioxidant effect (Botsoglou *et al.,* 2004), among several hundred of plants, Blomhoff (2004) found that oregano, followed by sage were ranked first and second to have anti-oxidant activity against oxidative stress. Also, oregano has a growth promoting and a hypolipidemia action (Ibrahim *et al.,* 2000). Sweet basil (*Ocimum basilicum L.*) with its high content of anethole, showed anti-microbial activity (Lachowicz *et al.,* 1998), also, the phenolic compounds such as rosmarinic acid in sweet basil has anti-oxidant activity (Jayasinghe *et al.,* 2003). and has a clear role in enhancing growth performance and lowering blood content of total lipids and cholesterol (Ibrahim *et al.,* 2000). It is considered beneficial to use a mixture of different sources of medicinal plants rather than a single source (Moleyar and Narasimham, 1992), the synergistic properties of different oils can keep the effective anti-microbial concentration of essential oils as low as possible due to characteristic flavors (Lee *et al.,* 2004), thus improving the anti-microbial activity in spite of low dosages.

Oligosaccharides as prebiotics are a class of carbohydrates that are not absorbed or digested in the small intestine of animals, but readily fermented by the intestinal microflora. This may result in changes in this flora, thereby increasing the number of beneficial micro-organisms, while repressing the harmful bacteria (Quigley, 2004). The phosphorylated mannan oligosaccharides, Bio-Mos®, derived from the outer cell wall of the yeast *Saccharomyces serivisae*, consist of a mannan component. The structure of the mannan resembles that of the carbohydrates on the animal gut wall. In theory, pathogenic, growth inhibiting microbes that normally adhere to mannans on the gut wall may instead bind to the mannan component of Bio-Mos®, so these pathogens flushed out from the upper gut, and do not attach to the mucosal receptors. Elimination of the pathogens would presumably enhance the health and growth of the animal. While, other sources of oligo-saccharides, is gluco-oligosaccharides act as substrates for 'desired' micro-organisms (Newman, 1994 and Huyghebaert, 2003). Providing Bio-Mos®, resulted in reduced mortality rate, improved feed conversion ratio (FCR) and similar daily weight gains (DWG) compared to oxytetracycline (Fonseca *et al.,* 2004). Both MOS and oxytetracycline induced longer villi, increased absorption area and caecal VFA's, moreover, decreased caecal pH compared to the control not medicated (Pinheiro *et al.,* 2004), while, Gidenne (1995) found no effect on DWG, FCR and caecal VFA's pattern when offered GOS® to rabbits fed low crude fiber diet.

The objective of this study was to evaluate the response of growing rabbits to some growth promoters as save alternatives to antibiotics.

MATERIAL & METHODS

The study was carried out during August-September months, at Animal Prod. Res. Inst. Station, ARC, Egypt. Seventy-6 week old New Zealand White rabbits were evenly sexed, weighed and individually caged to evaluate one of the following seven feed additives: 1: no supplement, 2: 0.5% acetic acid (96%), 3: 0.5% lactic acid (90%), 4: 0.5% a herb mixture of equal parts of sage+oregano+sweet basal, 5: 1.0% of the previous herb mixture, 6: 1.0 g GOS® (prebiotic)/ kg diet, and 7: 1.0 g Bio-Mos® (prebiotic)/ kg diet. the herb mixture included an equal parts of sage + oregano + sweet basil. GOS® is a glucan-oilgosaccharides, while, Bio-Mos® is a mannan oligosaccharide, both are products of Alltech Inc., USA. Additives studied were supplemented on the expense of total diet. Basal diet was formulated according to the NRC (1977) recommendation for growing rabbits. Ingredient and chemical composition of the basal diet are presented in Table (1). Total essential oils of each medicinal plant incorporated were conducted (Guenther, 1961). Rabbits were kept under managerial routine. Rabbitry air temperature and relative humidity were recorded daily. Growth trial lasted for 7 weeks, after which 3 rabbits of each treatment were fasted for 12 hours, and then slaughtered to complete bleeding. Blood plasma samples were collected for further determination of total protein, albumin, total lipids and total cholesterol While, plasma globulin was deduced as the difference between total protein and albumin Ileum samples were collected for histological study, absorption area was calculated (Fonseca *et al.,* 2004) as [villi height/ (villi height/crept depth)]. Volatile fatty acids (acetic, propionic and butyric) of the cecal content (HPLC; Kenaur-model) were determined. On slaughtering, pH of blood, cecum, and ileum (pH digital-pH meter) were measured. Hot carcass, giblets (heart+liver+kidneys+lungs), total edible parts (hot carcass+giblets+head) and abdominal fat percentages were proportioned to live weight of rabbits assigned for slaughter test. Data were subjected to a one-way analysis using SAS (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960).

Table 1. Ingredients and calculated chemical composition of the basal experimental diet

Ingredients	%	Calculated chemical composition	
Wheat bran	24.45	DE	2651 (kcal.kg^{-1}diet)
Barley	22.50	CP	17.09%
Soybean meal (44% CP)	19.25	CF	12.03%
Clover straw	17.80	Calcium	0.81%
Yellow corn	9.50	Phosphorus	0.54%
Molasses	4.00	Lysine	0.80%
Limestone	1.77	Methionine and Cystine	0.61%
Sodium chloride	0.30		
Vitamins and minerals mixture *	0.30		
DL-Methionine	0.13		
Total	100.0		

*Supplied per kg. of diet: 12000 IU vit.A; 2200 IU vit. D3; 10 mg vit. E; 2.0 mg vit. K_3; 1.0 mg vit. B_1; 4.0 mg vit. B_2; 1.5 mg vit. B_6; 0.0010 mg vit. B_{12}; 6.7 mg vit. PP; 6.67 mg vit. B_5; 0.07 mg B_8; 1.67 mg B_9; 400 mg Choline chloride; 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn;1. 67 mg Cu; 0.25 mg I and 0.033 mg Se.

RESULTS AND DISCUSSION

Throughout the seven-week growth trial, the recorded daily air temperatures were laid between (29–33°C), while relative humidity values were ranged between 50–63%. The total essential oils in oregano was 1.25%, in sage was 1.15%, while in sweet basil was 0.80%

1 – Growth performance

Table (2) illustrates the results growth performance criteria; initial live weight, total weight gain, feed intake and feed conversion ratio. It is clear that all suggested additives significantly (*p*<0.0.01) surpassed the control in total weight gain, especially with 0.5% herb mixture supplementation, also improved feed conversion ratio (*p*<0.0.05) was recorded. These results and those reported by Scapinello *et al.,* (1998) agree that a reduction in feed intake and improved in feed conversion ratio were attained when rabbits fed on diets with an incremental level of 0.5% of acetic acid up to 2.0%. Also, El-kerdawy (1996) found that fumeric, citric, and malic acids at the rated of 0.5% increased weight gain and return of rabbits. While, El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit weight gain, but decreased feed intake, moreover, worsened feed conversion ratio. It is worth noting that Tsiloyiannis *et al.,* (2001) found that lactic acid was the most effective organic acid to improve performance traits of pigs. This improvement in growth rate with organic acids in this study may be due to decreased pH of the cecum and ileum to levels not favorable for pathogens, also that it serves as substrates in the intermediary metabolites, increase gastric proteolysis and availability of some elements that complex with (Kirchgessner and Roth, 1988). Regarding the herb mixture, Ibrahim *et al.,* (2000) using sweet basil or oregano at the level of 0.5%, and again, Ibrahim *et al.,* (2002) using 0.5 or 1.0% sage found that such supplements significantly increased weight gain, feed intake, and improved feed conversion of rabbits. Khayyal (2006) found that 0.25 *vs.* 0.0, 0.5 or 1.0% of dried sage leaves was more effective in enhancing growth performance of rabbits. While, Botsoglou *et al.,* (2004) reported that dietary oregano essential oil (100 or 200 mg/kg diet) exerted no growth promoting effect on rabbits. It is obvious that the lower level of herb mixture (0.5%) gave the highest weight gain, and the highest absorption area value (Table 4), also compared with the higher level (1.0%) of herb mixture it gave higher weight gain and feed intake, in this regard, Lee *et al.,* (2004) considered that it is beneficial to keep the effective anti-microbial concentration of essential oils as low as possible due to characteristic flavors. Also the improvement with using a mixture may be due to synergistic properties of different oils (Moleyar and Narasimham (1992). Respecting prebiotics, our results conflict with those of Gidenne (1995) who reported no effect on weight gain or feed conversion when rabbits were offered GOS® to low crude fiber diet, but agree with Fonseca *et al.,* (2004) who found that providing Bio-Mos®, resulted in improved feed conversion ratio (FCR) and similar daily weight gains (DWG) compared to oxytetracycline. This may be attributed to that Bio-Mos® induced longer villi and increased absorption area compared to the control not medicated (Pinheiro *et al.,* 2004).

Table 2. Effect of studied feed supplements on growth performance

Variable / Treatment	Initial live weight (g)	Total weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	619±18	1128^{c}±22	4656±171	4.14^{b}±0.16
Acetic acid	617±21	1202^{b}±23	4459±111	3.71^{a}±0.05
Lactic acid	620±18	1224^{ab}±22	4564±88	3.73^{a}±0.04
Herb mix (0.5%)	621±18	1279^{a}±18	4843±145	3.79^{a}±0.11
Herb mix (1.0%)	616±19	1207^{ab}±30	4517±155	3.74^{a}±0.09
GOS®	619±24	1267^{ab}±21	4790±66	3.78^{a}±0.02
Bio-Mos®	619±20	1224^{ab}±26	4703±158	3.83^{a}±0.06
Significance	ns	**	ns	*

Means in the same column within each factor differently superscripted are significantly different.
ns: not significant, * : ($p<0.05$), and **:($p<0.01$),

2 – Plasma constituents

Results in Table (3) indicate that of the studied plasma constituents, only plasma total lipids and cholesterol were significantly affected by supplements evaluated. It is clear that 1.0% herb mixture and GOS® recorded the lowest total lipids and cholesterol, respectively. In this respect, Ibrahim *et al.,* (2000) found that sweet basil or oregano at the level of 0.5%, each significantly had a hypo-lipidemic effect. Also, the decrease in cholesterol level with increasing the level herb mixture may be due to that pure components of essential oils inhibit hepatic 3-hydroxy-3-methy glutaryl coenzyme A (HMG-CoA) reductase activity (Crowell, 1999), Which is a key regulatory enzyme in cholesterol synthesis, yet, Khayyal, (2006) reported a slight decrease in rabbits' plasma total lipids and cholesterol when fed on diets fortified with sage leaves at levels ranged between 0.25 and 1.0% in an incremental level of 0.25%. With prebiotic supplementation, Kannan *et al.,* (2005) reviewed that the decreased in blood cholesterol with prebiotic supplementation is a result to the cholesterol assimilation by *Lactobacillus*, as the prebiotic supplementation could enhance the lactobacilli count. *Lactobacilius spp.* are able to incorporate cholesterol into the cellular membrane of the organism. The decrease in plasma total lipids and cholesterol with organic acid supplementation may be due to the inhibition of lipogenesis in liver as reported by Fushimi *et al.,* (2006). Also results agree with El-kerdawy (1996) who found that plasma total lipids decreased, while total protein was not affected by fumeric, malic and citric acids supplementation at the rated of 0.5% of rabbit diets.

Table 3. Effect of studied feed supplements on some plasma constituents

Variable / Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Cholesterol (mg/dl)
Control	6.81±0.20	5.02±0.48	1.79±0.37	488^{ab}±14	104^{a}±4.0
Acetic acid	7.69±0.51	4.72±0.36	2.97±0.26	456^{abc}±28	91^{b}±2.3
Lactic acid	7.77±0.42	5.05±0.05	2.72±0.37	406^{abc}±19	93^{ab}±4.0
Herb mix (0.5%)	7.18±0.41	5.21±0.28	1.97±0.12	384^{bc}±17	95^{ab}±1.2
Herb mix (1.0%)	7.90±0.29	5.14±0.38	2.75±0.65	360^{c}±53	90^{b}±5.4
GOS®	6.92±0.16	4.43±0.22	2.49±0.30	478^{ab}±38	72^{c}±3.5
Bio-Mos®	7.20±0.30	4.61±0.16	2.59±0.34	508^{a}±34	95^{ab}±3.6
Significance	ns	ns	ns	*	*

ns: not significant,
*:means in the same column within each factor differently superscripted are significantly different ($p<0.05$)

3 – Villi measurements and absorption area

Results in Table (4) reveal that herb mixture at the level of 0.5%, followed by both prebiotic sources increased (p<0.01), both villi height and crept depth, and absorption area (p=0.06) compared with control. In this connection, Pinheiro *et al.,* (2004) reported that Bio-Mos® at the rate of 1.0, 1.5 and 2.0 kg/ton rabbit diet resulted in a significant increase in villi height, which in turn translated into a numeric increased absorption area of the intestine over that reported for the control group. While, Mourão *et al.,* (2004) reported that fructo-oligosaccharides had no effect on villi measurements when added to rabbit diet. Elimination of the pathogens would presumably enhance the health and of the gut condition as reported by (Newman, 1994 and Huyghebaert, 2003).

Table 4. Effect of studied feed supplements on villi measurements (µm) and absorption area (10^3µ m^2)

Variable / Treatment	Villi height	Crept depth	**Villus height** Crept depth	Absorption Area
Control	$635^{c}\pm10$	$160^{c}\pm3.9$	3.98±0.07	166.3±4.19
Acetic acid	$650^{c}\pm13$	$172^{bc}\pm5.9$	3.82±0.11	168.8±7.89
Lactic acid	$665^{bc}\pm19$	$167^{bc}\pm8.0$	4.04±0.12	163.8±8.85
Herb mix (0.5%)	$740^{a}\pm21$	$203^{a}\pm7.9$	3.72±0.19	198.8±11.1
Herb mix (1.0%)	$689^{abc}\pm26$	$187^{abc}\pm12.1$	3.79±0.18	183.8±12.1
GOS®	$724^{ab}\pm23$	$192^{ab}\pm10.9$	3.85±0.14	183.8±8.65
Bio-Mos®	$721^{ab}\pm25$	$193^{ab}\pm11.3$	3.82±0.17	197.5±13.6
Significance	**	**	ns	ns

ns: not significant,
**:means in the same column within each factor differently superscripted are significantly different (p<0.01)

4 – Cecal volatile fatty acids concentration and pH of cecum, ileum, and blood

Results in Table (5) indicate that, any of the studied supplements had a significant effect on volatile fatty acids production (acetic, propionic and butyric) or pH of the cecum, ileum or blood (p=0.06). El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit cecum pH. Mourão et al., (2004) reported that fructo-oligosaccharides had no effect on VFA production when added to rabbit diet (0.36g/kg diet). While, Pinheiro *et al.,* (2004) reported that Bio-Mos® at the rate of 1.0, 1.5 and 2.0 kg/ton rabbit diet resulted in a significant increase in VFA's and a significant decrease in cecal pH. It is worth noting that an important tool to qualitatively evaluate the microbial activity in the intestine is to measure the volatile fatty acids concentrations as end-products of microbial fermentation (Bellier and Gidenne, 1996 and Pinheiro *et al.,* 2004). Also, that the rabbit is unusual in that butyric acid is a more important VFA than propionic acid as an end product of cecal fermentation, this may be important in preventing enteritis (Cheeke, 1987).

Table 5. Effect of studied feed supplements on cecal volatile fatty acids concentration (mmol.1000 ml^{-1}) and pH of cecum, ileum, and blood

Variable / Treatment	Acetic acid	Propionic acid	Butyric Acid	pH cecum	PH ileum	pH blood
Control	46.5±0.6	21.9±2.3	29.7±1.6	6.17±0.07	7.36±0.03	7.32±0.06
Acetic acid	45.2±2.0	22.2±0.9	28.4±0.5	6.05±0.16	7.24±0.05	7.39±0.05
Lactic acid	48.4±0.8	20.0±0.1	27.4±1.0	5.97±0.11	7.31±0.09	7.50±0.02
Herb mix (0.5%)	47.8±0.2	22.9±0.7	27.2±1.9	6.35±0.15	7.27±0.06	7.39±0.10
Herb mix (1.0%)	46.7±2.8	25.0±3.4	25.9±2.1	6.26±0.09	7.35±0.15	7.56±0.02
GOS®	47.8±0.9	23.5±0.2	26.0±0.8	6.20±0.22	7.38±0.18	7.50±0.03
Bio-Mos®	44.3±1.3	22.1±3.0	29.6±1.5	6.30±0.18	7.48±0.07	7.49±0.02
Significance	ns					

ns: not significant

5 – Carcass characteristics of rabbits

Results in Table 6 indicate that relative to the slaughter weight, hot carcass, giblets, and total edible parts percentage, were not significantly affected by supplements under study, while, abdominal fat % was significantly decreased as summer reared rabbit diets were fortified with acetic acid or the 1.0% herb mixture. El-Allawy (2001) found that citric acid (5.0 g/kg diet) had no effect on rabbit dressing %. Ibrahim *et al.,* (2000) found that sweet basil or oregano at the level of 0.5% increased significantly dressing and giblets %.Khayyal (2006) reported higher total edible parts of rabbit carcass % as sage leaves were included in the diet at 0.25% compared to the control or those supplemented with 0.50 or 1.0%.Mourão *et al.,* (2004) reported that fructo-oligosaccharides had no effect on weights of liver when added to rabbit diet (0.36g/kg diet).

Table 6. Effect of studied feed supplements on some carcass characteristics (%)

Variable / Treatment	Slaughter weight (g)	Hot carcass	Giblets	Total edible	Abdominal fat
Control	1778±53	50.9±1.2	4.5±0.6	61.2±1.5	0.72^{a}±0.04
Acetic acid	1884±39	52.3±0.8	4.8±0.2	62.7±0.7	0.46^{b}±0.07
Lactic acid	1886±49	51.2±1.6	4.6±0.1	61.2±1.6	0.61^{a}±0.04
Herb mix (0.5%)	1921±38	52.4±1.2	4.6±0.2	62.5±1.5	0.63^{a}±0.04
Herb mix (1.0%)	1829±15	52.4±0.6	5.3±0.3	63.3±0.3	0.46^{b}±0.03
GOS®	1838±24	52.5±0.3	4.9±0.2	63.2±0.1	0.63^{a}±0.02
Bio-Mos®	1900±30	50.2±0.3	4.6±0.6	60.2±0.5	0.71^{a}±0.03
Significance	Ns	ns	ns	ns	**

ns: not significant,
**: Means in the same column within each factor differently superscripted are significantly different (p<0.01)

CONCLUSION

The current study proved that these additives were effective to correct some of deleterious effects of high ambient temperatures on rabbit growth performance and some biological functions.

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