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Effect of Using Echinacea Extract as Immuno-stimulating Additive on Milk Yield Traits, Immunity and Udder Health of Zaraibi Goats

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ABSTRACT

This study was performed to investigate the effect of using Echinacea extract, as immuno-stimulating feed additive, on milk production, milk composition, udder health and immune response of Zaraibi goats. Milk samples were collected at mid-lactation from half udder of Zaraibi goats flock. Animals with high somatic cell counts (SCC > 1.5×10^{-6}) were diagnosed as sub-clinically infected with mastitis. Forty lactating does were chosen out of the flock and grouped equally (20 does in each) according to health status of the udder. Each group was divided into 2 sub- groups (A and B) as healthy and (C and D) as infected. Immulant pills containing Echinacea purpurea extract were given orally once daily over two weeks to the does in groups B and C (525 mg/day) and twice daily to the does in group D (1050 mg/ day). Milk and blood samples were collected from all animals before, during and after the treatment.

Results showed that the overall LS mean of log SCC in infected does (6.2) was significantly higher than that in non-infected one (5.8). Log SCC decreased significantly during supplementation with Echinacea extract. LSM of milk protein, total solid and solid not fat% were significantly affected by parity number with the highest levels observed in the sixth parity. LSM of fat, protein, total solid and solid not fat % were significantly higher in milk of does received different doses of Echinacea extract (B, C and D) than those of the control group (A). However, their levels remained within the normal ranges recorded for goats.

Serum albumin and γ-globulin levels were significantly affected by health status of udder half and supplementation with Echinacea extract. All treated groups (B, C and D) showed higher globulin level during the experimental period than the non-treated group. Serum total protein and globulin levels increased significantly at the 2nd week during the treatment. Electrophoresis pattern of goats' serum total protein showed that changes of serum γ-globulin levels were parallel to those of serum total protein and globulin.

The use of Echinacea extract as immuno-stimulating feed additive to achieve beneficial changes in health of the mammary gland, improves goat immune response, and improves the hygienic and health- promoting quality of milk is recommended. The results suggest the use of the lower level of Echinacea extract (525 mg/day/ head) in order to get better economic return.

Key words: Mastitis, parity, Echinaccea purpurea, serum total protein, globulin, γ-globulin.

INTRODUCTION

Mastitis is one of the most costly diseases in the dairy industry (Khan *et al*., 1991). The major components of the economic losses caused by mastitis are: reduced

milk yield and quality، increased labor and veterinary costs, possible discarded of milk and reduced longevity of milking goats (Blood and Radostits, 1989 and Ullah, 2004). Moreover, high mortalities of kids born to goats with mastitis make the disease significantly affecting economics of goat industry (Addo *et al.*, 1980). Mastitis is caused by several species of common bacteria, fungi, mycoplasma and algae. Sub-clinical infections are those of no visible changes occur in the appearance of milk or udder (Tyler and Cullor, 1990). Economically, sub-clinical mastitis is more important than clinical mastitis. It usually precedes the clinical form for its longer duration, difficult to be detected, adverse effects on milk quality, production and constitutes a reservoir of microorganisms that lead to infection of other animals within the herd (Hamed *et al*., 1993; Urech *et al*., 1999; Shearer and Harris, 2003).

The determination of milk somatic cell counts (SCC) is widely used to monitor udder health and milk quality. During inflammation, major increases in SCC take place due to influx of neutrophils into milk. Jones (2006) reported that the higher the SCC, the greater in risk of raw milk contamination with pathogens and antibiotic residuals. Increasing of SCC reduce the suitability of raw milk for manufacturing and processing into products for human consumption (Khan and Khan, 2006).

Zaraibi goats are the most pronounced dairy goat among the local breeds in Egypt. It considered of high genetic potential as a dairy and prolific breed (Aboul-Naga *et al*., 1993). Their milk production averaged 249 kg in a lactation period of 210 days (El-Saied *et al*., 2007). Most of goat milk is used by dairy industry for cheese making to cover the little demand of the domestic market. The cheese-making process is largely affected by milk quality, especially casein percentage (El-Saied *et al*., 2003). Consumers do not only look for fresh and tasty milk or cheese, but also safe and healthy. The over use of antibiotics in human and animals led to rapid rise in the number of bacteria strains resistant to majority of antibiotics, which makes infection harder to control (Tan *et al*., 2000). The withdrawal of antibiotics from animal production is the reason to seek for alternative solutions. One of this solutions is concerned with making use of the organisms own defense system. The use of officinal plants to enhance the immune system has been studied in goats. Reklewska *et al*. (2004) reported that Echinacea purpurea extract reduced mammary infections and SCC in goats' milk by increasing lactofferin secretion, which is anti-bacterial, anti-viral and immuno-stimulating compound. Echinacea purpurea is a popular medicinal herb which activates the body's immune system and increases the chance of fighting most diseases (Burger *et al*., 1997 and Chang 2000). Echinacea's pharmacological activity depends on combined activity of several constituents (Bauer and Wagner, 1991), mainly the alkamides, caffeic acid derivatives and polysaccharides. A variety of Echinacea alkamides have been shown to stimulate immune cell activity and inhibit enzymes involved in the production of inflammatory mediators (Muller- Jakic *et al*., 1993 and Goel *et al*., 2002). Caffeic acid derivative has been shown to stimulate the activity of the immune cells to exhibit antiviral and antioxidant activity (Cheminat *et al*., 1988, Bauer, 1999 and Sloley *et al*., 2001) and inhibit hyaluronidase enzyme which involved

in infection and inflammation (Facino *et al*., 1993). Moreover, polysaccharides stimulate the activity of the immune cells to exhibit anti- inflammatory activity (Tubaro *et al*., 1987 and Roesler *et al*., 1991).

The present study aimed to investigate the effect of using Echinacea extract as immuno-stimulating additive on milk production, animal health status and immune response of Zaraibi goats.

MATERIALS AND METHODS

Animal management:

The experimental work was carried out at Sakha Station, Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Kafre El-Sheikh Governorate, Egypt.

Prior to the experimental work, milk samples were collected at mid-lactation from half udder of all Zaraibi goats in the flock (82 does). All lactating animals were screened and scored for mastitis. Animals free from clinical abnormalities and giving apparently normal milk but showed high somatic cell counts (SCC > 1.5×10^{-6}) were diagnosed as sub-clinically infected with mastitis (El-Saied *et al*., 2003). Out of the flock, 40 animals were grouped equally (20 does each) according to their health status (healthy or infected). The healthy group was divided into 2 sub-groups (A and B); while infected animals were sub-grouped into groups C and D. Immulant pills (Mepaco-Egypt) containing Echinacea purpurea extract were given orally once daily (3 pills) over two weeks to goat in groups B and C and twice daily to goats in group D (6 pills/ day). Each pill of the Immulant contains 175 mg of dry extract. The given dose was made according to Reklewska *et al*. (2004). Animals were fed according to nutrient requirements of goats (NRC, 1981). Water and minerals blocks were available all the time.

Milk and Blood samples:

Blood and half udder milk samples were collected from all lactating does before treatment (week 0). During the treatment, samples were collected weekly for 2 weeks (week 1 and 2), then at weeks 3 and 4 after the end of treatment.

Blood samples were left to clot at room temperature for at least 4 h. The clots were removed and sera were cleared by centrifugation at 1500×g for 20 min and stored at -20 ºC until used for total protein, albumin, globulin and electrophoretic analysis.

Bacteriological analysis and SCC determination:

Bacteriological examination of milk samples was performed for half udder of infected goats (groups C and D). Bacteriological tests were carried out using the Standard Plate Count (SPC) according to Houghtby *et al*. (1992) and APHA (1993). The presumptive coli form bacterial count was counted using Maconkey agar media (Difco, Detroit, MI). The presumptive Streptococcci groups causing mastitis (*st. agalactiae*, *st. dysagalactiae* and *st. uberis*) were enumerated on modified Edward's media (Oxford, Hampshire, England) and added blood to media for identification of blood hymolysis bacteria. The presumptive *Staphylococcus aurues* and other types of

Staphylococcus were counted on Barid Parker agar media (Oxford, Hampshire, England) with sheep blood for appearance of hymolosis species. Blood agar (Nutrient agar media plus sheep's blood) was used for enumeration of *Bacillus spp*. Blood agar (Nutrient agar media plus sheep's blood) with potassium telluerite was used for presumptive *Corynebacterium* species. All plates were incubated at 37± 2^{o}C and examined after 24 and 48 hours. These selective and differential media were chosen for the isolation and identification of mastitis inducing pathogens according to Collins and Patricia (1976) and APHA (1993). The results were expressed as colony forming unit CFU/ ml of milk.

Milk composition:

Milk composition of milk samples were estimated with infra-red spectroscopy (Milko-Scan 133B; N. Foss Electric, DK 3400 Hillerod, Denmark). SCC for each milk sample was determined by the fluoro-opto-electronic method (Fossomatic 5000; Foss Electric apparatus, 3400 Hillerod, Denmark) 24 h post collection following the rules of the International Dairy Federation (1984) and the machine was calibrated for goat milk. Original scales of SCC values were transformed to its corresponding logarithmic form according to Ali and shook (1980) to meet the characteristics of hypothesis testing.

Biochemical analysis:

Total serum protein and albumin levels were measured using Stanbio kit (Catalog No. 0250 and 0285, respectively) used for quantitative colorimetric determination of total protein and albumin (Gornal *et al*., 1949 and Dumas and Biggs, 1972, respectively). Globulin levels were calculated from data recorded for total protein and albumin.

Serum protein fractionation:

Serum samples were collected at weeks (0, 1, 2, 3 and 4) from 3 goats selected randomly of each group (A, B, C and D) to be used for fractionation of serum proteins. Electrophoretic separation of serum protein to 4 bands (albumin and α, β and γ-globulin) was applied according to Nils (1983). Quantization of different fractions was performed using Gelman DCD 16 digital computing densitometer. Results were performed as percentages.

Statistical model:

Data were analyzed to study the effect of udder half, health status, treatment with Echinacea extract and parity as fixed effects and animal as random on log SCC_{10}, milk yield, milk composition (fat, protein, lactose, total solid and solid-not fat %), serum total protein, albumin, total globulin and α, β and γ-globulin using the MIXED procedure of SAS (SAS, 1996).

RESULTS AND DISCUSION

Results of analysis of variance of the factors affecting SCC and milk composition traits of Zaraibi goats are presented in Table 1.

LSM of log SCC (6.2) in milk samples collected from infected groups was significantly higher (P> 0.0001) than that for non-infected groups (5.8) (Table 4). Log

SCC was affected by parity number, where goats in the sixth parity exhibited significantly higher log SCC (*P*> 0.0001) than other parities. Log SCC recorded in the present study was higher than that reported by El- Saied *et al*. (2003) in infected and non-infected Zaraibi goats (6.2 vs. 5.7). The same author reported that the fifth parity had the highest log SCC. The increase in SCC with number of lactations was also reported in dairy ewes (Bergonier *et al*., 1996; Bencini and Pulina, 1997). In contrast, Nudda *et al*. (2003) reported that SCC did not differ significantly among parities. The increase of SCC with parity number may be due to the increase in the prevalence of sub-clinical mastitis as age advances.

Table 1: Analysis of variance for log SCC and milk composition traits in Zaraibi goats.

Source of variance	df	Log_{10} SCC	Fat %	Protein %	Lactose %	Total solid %	Solid not fat %
U. half	1	.55*	2.0^{NS}	0.1^{NS}	2.2^{NS}	0.03^{NS}	0.6^{NS}
HS	1	61.7***	4.7^{NS}	1.8^{NS}	0.4^{NS}	2.9^{NS}	0.5^{NS}
Treat (HS)	2	4.5*	8.2***	6.2**	4.5*	13.0***	8.8***
Week (HS* treat)	16	5.2***	2.6***	3.2***	3.2***	2.2***	2.4**
Parity number	7	5.8***	3.2**	9.8***	1.7^{NS}	4.2***	3.9***
TB	3	3.0*	1.4^{NS}	8.8***	1.8^{NS}	2.4^{NS}	6.3***

U. half= udder half (left or right); HS = health status of the udder; treat= different animal groups [control group (A), goats received 525 mg/ day of Echinacea extract (groups B and C) and goats received 1050 mg/ day of Echinacea extract (group D)]; TB= type of birth. NS = non significant, * = significant at P> 0.05, ** = significant at P> 0.001, *** = significant at P> 0.0001

Bacterial types isolated:

Frequency of different bacterial types isolated from milk samples of goats infected with sub-clinical mastitis (groups C and D) are presented in Table 2. Eight types of microorganisms were found in milk samples of the infected groups. Most pathogens isolated during the experimental period in goats of the infected groups (C and D) were *Staphylococcus aureus* (6.0 and 3.2%, respectively), *Streptococcus dysgalactiae* (3.6 and 3.2%, respectively), *Streptococcus Uberis* (0 and 1.1%, respectively) and *Coliform spp* (4.8 and 4.3%, respectively). In addition, others as Staphylococci spp. (7.2 and 7.5%), *Corynebacteria spp*. (16.9 and 15.1 %) and *Bacillus* (12.1 and 11.8 %) were also

isolated from milk samples of goats in groups C and D, respectively. During the bacteriological examination, samples infected with triple minor pathogens were excluded from the statistical analysis. El- Saied *et al*. (2003) recorded eight types of microorganisms in the same breed, where the most dominant bacterial types were *Staphylococci spp*. (18.9 %), *Streptococcus dysgalactiae* (2.9 %) and *Corynebacteria* (1.7 %), and they also recorded 11.4 % for *Bacilli* as a minor pathogen. Similar bacterial isolates were reported for goat milk worldwide. Adwan *et al*. (2005) reported that most pathogens isolated from milk samples of goats in the north of Palestine were *Staphylococci* (68.3 %), which is the more prevalent bacteria that can cause sub-clinical mastitis (*aureus*, *epidermidis* and *saprophyticus*). They also reported 4 % for Bacillus spp. Lerondelle *et al*. (1992) detected *S. aureus* (2%) and coagulase negative *Staphylococci* (23%) in France. However, Kapur *et al*. (1992) and Allore (1993) reported that S*taphylococcus aureus* was the predominant organism responsible for the most clinical mastitis cases in cow. The prevalent of sub-clinical mastitis microorganisms differs among regions, which might be due to the differences in environment, management conditions and methodological approach used.

Table 2: Frequency of different bacterial types isolated from milk samples of Zaraibi goats.

Type of pathogen	Group (C)			Group (D)		
	Number	%	Average (CFU/ ml)	Number	%	Average (CFU/ ml)
Major pathogens:-						
Str. agalactiae	0	0	----	1	1.1	440
Staph. aureus	5	6.0	740	3	3.2	667
Str. dysgalactiae	3	3.6	1853	3	3.2	1910
Str. Uberis	0	0	----	1	1.1	600
Coliform spp	4	4.8	1928	4	4.3	2833
Minor pathogens:-						
Other Staphylococci spp.	6	7.2	1833	7	7.5	676
Corybacteria spp.	14	16.9	801	14	15.1	938
Bacillus spp	10	12.1	1152	11	11.8	785

Somatic cell count (SCC):

Results of the present study showed that supplementation with Echinacea purpurea extract affects significantly SCC in infected goat groups (C and D), which treated with different doses of Echinacea extract (525 and 1050 mg/ day, respectively) with no differences observed between the two groups (Table 4). Figure 1 shows that before the treatment (week 0) , log SCC in milk samples of infected does groups (C and D) (6.4 and 6.5, respectively) were significantly higher than in healthy groups A and B (5.7 and 5.8, respectively). During the 1st week of supplementation with Echinacea extract, log

SCC in infected groups (C and D) decreased significantly by 9.4 and 6.2 %, respectively, than its initial levels before the treatment (week 0). However, two weeks after terminating the treatment (week 4), log SCC slightly increased reaching levels close but still lower than those before the treatment (Figure 1). The decrease in log SCC in infected does during the treatment, prove the effect of Echinacea extract to stimulate goats' own defense system. In agreement, Reklewska *et al*. (2004) reported that Echinacea purpurea extract stimulate the goat's immune system effectively, acting specifically on the secretion of lactoferrin. In other studies, Echinacea extract has proven the ability to activate the immune system (Burger *et al*., 1997 and Chang, 2000); enhanced phagocytic activity of polymorphonuclear neutrophils (Chang, 2000); stimulated macrophages cells to produce more cytokines and interferone (Burger *et al*., 1997). In contrast, Dymnicka *et al*. (2003) observed no significant changes in milk SCC in cows given 300 g/ animal of dried whole Echinacea purpurea plant over 3 weeks. The differences between the present result and that reported by Dymnicka *et al*. (2003) may be due to that the amount of the dried plant (300 g/ animal), which given to cows, was not active as the pharmacological form used.

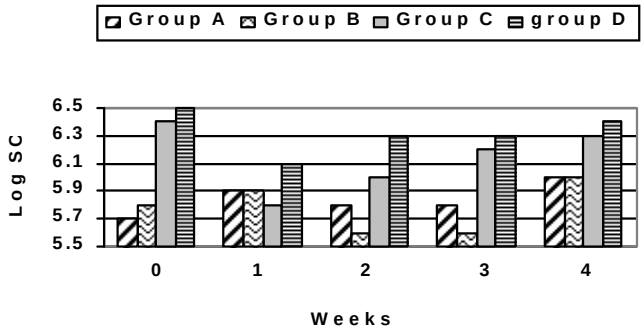


Figure 1: Log SCC in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) of goat in groups A, B, C and D.

Milk yield:

Milk yield was not significantly affected by health status of the udder or supplementation with Echinacea extracts, but it was significantly affected ($P > 0.0001$) by both parity number and type of birth (Table 3). Goats showed the highest milk yield at the third and the fourth parity (1.0 and 1.1 kg/ day, respectively).

However, (Table 4), shows slight increase in milk yield (0.8 kg) in groups (B, C and D) receiving different doses of Echinacea extract (525, 525 and 1050 mg/ day, respectively) compared to the control (0.7 kg). During the treatment, Figure 2, milk

Table 3: Analysis of variance for milk yield, serum total protein (STP), albumin (Alb.), globulin (Glob.) and α, β and γ-globulin in Zaribi goats.

Source of variance	df	***F* values and significance**						
		Milk yield	STP	Alb.	Glob.	**α** glob.	**β** glob.	**γ** glob.
HS	1	0.4 ^{NS}	1.3 ^{NS}	3.9 *	0.3 ^{NS}	0.2 ^{NS}	8.3 *	11.0 *
Treat (*HS)	2	1.2 ^{NS}	1.3 ^{NS}	0.9 ^{NS}	0.8 ^{NS}	1.6 ^{NS}	2.4 ^{NS}	0.4 ^{NS}
Week (HS* treat)	16	0.8 ^{NS}	8.2 ***	6.0 ***	7.5 ***	1.3 ^{NS}	1.6 ^{NS}	3.0 ^{NS}
Parity number	7	8.7 ***	1.6 ^{NS}	1.6 ^{NS}	2.1 *	___	___	___
Type of birth (TB)	3	4.8 **	0.2 ^{NS}	0.6 ^{NS}	0.2 ^{NS}	___	___	___

U. half= udder half (left or right), HS (health status of the udder); treat: (control group (A), goats received 525 mg/ day of Echinacea extract (groups B and C) and goats received 1050/ day of Echinacea extract (group D). NS = non significant, * = significant at P> 0.05,
** = Significant at P> 0.001, *** = Significant at P> 0.0001

yield was significantly higher in the first weak (0.9 kg/ day) in group (C) than groups A, B and D (0.7 kg/ day). While, at the second week goats in groups (B and D) showed significantly higher milk yield (1.0 and 0.9 kg/ day) than group C (0.7 kg/ day). Milk yield decreased two weeks after terminating of the treatment reaching levels close to that of the control group (A). Gonzalo and Sanchez (2002) and Kifaro, *et al*. (2009) reported that milk yield was negatively affected by health status of the udder in dairy ewes and goats, respectively. They added that the increase in somatic cell count is not always associated with a decrease in milk production. Wilson *et al*. (1995) reported that lowering milk production together with the high SCC could be due to the advance of lactation rather than the high SCC in goats. Reklewska *et al*. (2004) reported that addition of Echinacea extracts did not significantly affect milk yield increase in somatic cell count is not always associated with a decrease in milk production. Wilson *et al*. (1995) reported that lowering milk production together with the high SCC could be due to the advance of lactation rather than the high SCC in goats. Reklewska *et al*. (2004) reported that addition of Echinacea extracts did not significantly affect milk yield

Table 4: Least square means (± SE) of log SCC, milk yield (kg) and milk composition traits as affected by udder half, health status of the udder, treatment and parity in Zaraibi goats.

Effect	Log SCC	Milk yield Kg/ day	Fat %	Protein %	Lactose %	Total solid %	Solid not fat %
Uhalf:			NS	NS	NS	NS	NS
Right	6	___	3	2.7	3.9	10.3	7.2
	± 0.04 [a]		± 0.10	± 0.10	± 0.04	± 0.05	± 0.10
Left	6.1		3.1	2.7	3.8	10.3	7.8
	± 0.04 [b]		± 0.10	± 0.10	± 0.04	± 0.05	± 0.10
HS:		NS	NS	NS	NS	NS	NS
	5.8	0.8	3	2.6	3.8	10.2	7.1
Healthy	± 0.04 [a]	± 0.04	± 0.10	± 0.10	± 0.10	± 0.05	± 0.10
	6.2	0.8	3.2	2.7	3.8	10.4	7.2
Infected	± 0.04 [b]	± 0.04	± 0.10	± 0.10	± 0.04	± 0.05	± 0.10
Treat (HS):		NS					
A	5.9	0.7	2.9	2.5	3.8	9.8	6.9
	± 0.10 [a]	± 0.10	± 0.10 [a]	± 0.10 [a]	± 0.1 [a]	± 0.05 [a]	± 0.10 [a]
B	5.8	0.8	3.2	2.7	3.9	10.5	7.3
	± 0.10 [a]	± 0.10	± 0.10 [b]	± 0.10 [b]	± 0.1 [b]	± 0.05 [b]	± 0.10 [b]
C	6.1	0.8	3.1	2.7	3.9	10.4	7.3
	± 0.04 [b]	± 0.04	± 0.10 [b]	± 0.10 [b]	± 0.1 [ab]	± 0.05 [b]	± 0.10 [b]
D	6.3	0.8	3	2.7	3.7	10.3	7.1
	± 0.10 [b]	± 0.10	± 0.10 [b]	± 0.10 [b]	± 0.1 [a]	± 0.05 [b]	± 0.10 [cb]
Parity number:					NS		
1	6	0.6	3.5	2.5	3.8	10.6	7
	± 0.10 [a]	± 0.02 [a]	± 0.20 [a]	± 0.14 [a]	± 0.10	± 0.30 [a]	± 0.20 [a]
2	5.9	0.8	3.1	2.4	3.9	10	6.9
	± 0.10 [a]	± 0.02 [a]	± 0.10 [b]	± 0.10 [a]	± 0.10	± 0.10 [b]	± 0.10 [a]
3	5.9	1.04	3	2.4	3.8	9.9	6.9
	± 0.10 [a]	± 0.04 [b]	± 0.10 [b]	± 0.10 [a]	± 0.10	± 0.10 [b]	± 0.10 [a]
4	5.8	1.1	3	2.6	3.9	10.2	7.2
	± 0.10 [ab]	± 0.10 [b]	± 0.10 [b]	± 0.10 [ab]	± 0.10	± 0.10 [ab]	± 0.10 [ab]
5	6	0.9	2.8	2.8	3.7	10	7.2
	± 0.10 [ac]	± 0.10 [ab]	± 0.10 [bc]	± 0.10 [abc]	± 0.10	± 0.20 [a]	± 0.10 [ab]
6	6.5	0.4	3.3	3.6	3.7	11.2	7.9
	± 0.10 [abc]	± 0.20 [abcd]	± 0.20 [ab]	± 0.10 [bd]	± 0.10	± 0.30 [ac]	± 0.20 [b]
7	6.1	0.9	2.9	2.5	3.9	9.9	7
	± 0.10 [abc]	± 0.10 [abc]	± 0.10[bc]	± 0.10 [ad]	± 0.10	± 0.10 [b]	± 0.10 [a]
8	6.1	0.7	3.1	2.6	4	10.4	7.2
	± 0.10 [a]	± 0.10 [abc]	± 0.10 [bc]	± 0.10 [a]	± 0.10	± 0.20 [abd]	± 0.10 [ab]

NS= non significant. a, b, c, d= Means with the different letters are significantly different at P> 0.05

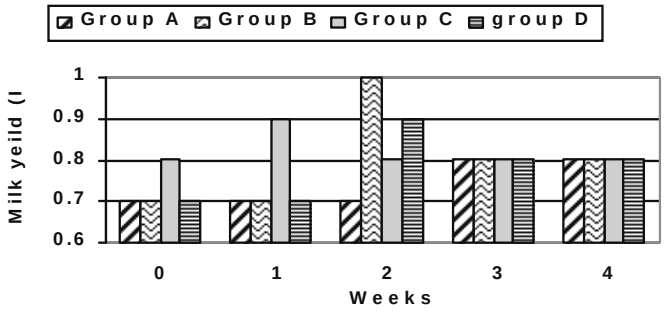


Figure 2: Milk yield (Kg) before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goat of groups A, B, C and D.

Milk composition:

Results of the analysis of variance of milk composition showed that fat, protein, lactose, total solid and solid not fat% were not significantly affected by infection status of udder (Table 1). In contrast, Kifaro, *et al*. (2009) reported that sub-clinical mastitis causes a significant decrease in milk butter fat and an increase in milk protein of dairy goat in Tanzania. Moreover, they reported insignificant changes in milk lactose, although it tended to decrease with the increase of mastitis severity. Jones (2006) reported that fat and lactose% were lower in case of infected udder than in non-infected one. LSM for milk protein, total solid and solid not fat% were significantly affected by parity number (Table 4), where the highest levels were observed at the sixth parity. However, milk fat% was significantly high at the 1st parity than other parties. Falagan and Mateos (1996) found that the highest levels of fat, protein, and solid not fat were reached around the 6th lactation. In contrast, Fermandez *et al*. (2008) reported that the levels of fat, protein and solid not fat were homogenous across the seven lactations. Supplementation with Echinacea extract with different doses significantly improved all milk composition traits, fat, protein, total solid and solid not fat %, (Table 4), compared to the control group. Meanwhile, milk lactose% was significantly higher in groups B and C (3.9%), which received 525 mg/ day of Echinacea extract than in groups A and D (3.8 and 3.7%), respectively. Before the treatment (week 0), fat, protein, lactose, total solid and solid not fat% were significantly higher in groups B, C and D than those in group A (Figure 3, 4, 5, 6 and 7). Milk fat% (Figure 3) showed a significant increase during the 1st week of the treatment in group B (3.5) and at the 2nd week in group D (3.4) than its initial level (3.1 and 3.0), respectively. Two weeks after the end of the treatment, milk fat% decreased in groups B, C and D reaching levels slightly higher than in group A. Meanwhile, milk protein did not significantly changed during the treatment in all groups (Figure 4). Lactose% (Figure 5) increased in all groups (A, B, C and D) at the 1st week of the experiment and remained high during the second week in

the treated groups (B, C and D). Reklewska *et al*. (2004) reported a significant decrease in milk protein and an increase in fat content during the treatment with Echinacea extract. Although, milk fat, protein, total solid and solid not fat % were significantly affected by supplementation with Echinacea extract. Their levels still within the normal ranges reported for different breeds of dairy goats. Fermandez *et al*. (2008) reported mean percentages of fat, protein, lactose, total solids of 3.7, 2.7, 4.5 and 11.9%, respectively in Mexico goats. Protein values have been estimated in the range of 2.6-4.3% (Alsina *et al*., 2002 and Salama *et al*., 2003). Lactose levels detected in the present study were lower than that reported by Buxade and Caballero, 1996 (4.4- 4.7%). Meanwhile, total solid was within the range reported by Salama *et al*., 2003 and Sanchez *et al*., 2005).

Serum total protein, globulin and γ- globulins:

Analysis of variance of factors affecting serum total protein, albumin, globulin and electrophoretic patterns of total serum protein (α, β, γ-globulins) are presented in Table 3. Results showed that serum albumin, β and γ-globulin levels were significantly affected by health status of the udder ($P > 0.05$). Table 5 shows that infected goat groups exhibited higher serum albumin and γ-globulin (5.3 g/ dl and 22.5%, respectively) than the non-infected groups (5.1 g/ dl and 19.6%, respectively). The increase in globulin levels was associated with a decrease in serum β-globulin levels, where its level was significantly lower (13.3%) in infected does than in non- infected ones (15.5%)

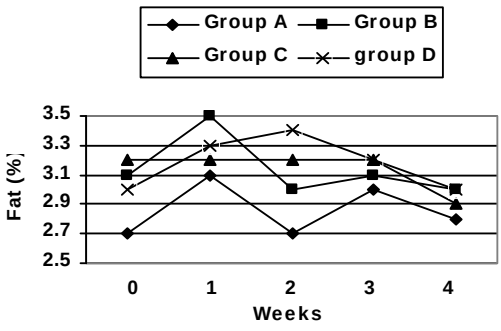


Figure 3: Fat (%) in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goat of groups A, B, C and D.

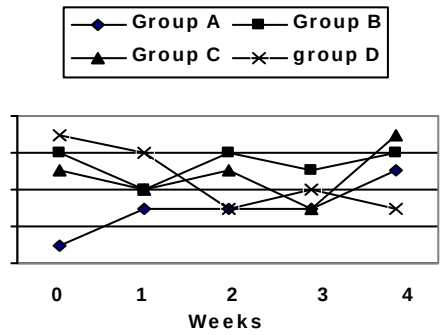
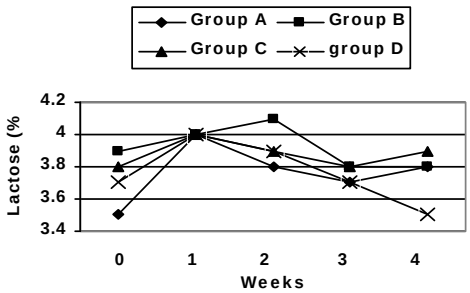


Figure 4: Protein (%) in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goat of groups A, B, C and D.



igure 5: Lactose (%) in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goat of groups A, B, C and D.

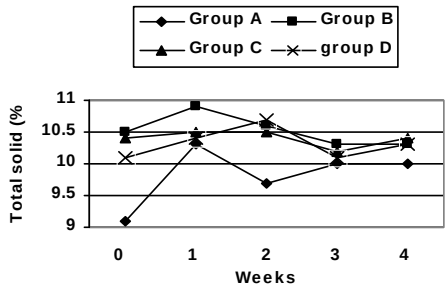


Figure 6: Total solid (%) in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goats of groups A, B, C and D.

Ohtsuka *et al*. (2006) reported that serum total protein, albumin and γ-globulin were significantly higher in cows infected by inflammatory disease. Serum albumin and γ-globulin levels were significantly higher in infected groups (C and D) supplemented with Echinacea extract than in A and B groups (Table 5). Although globulin levels in blood serum were not significantly differed between goats in different groups, all treated groups (B, C and D) showed numerically higher globulin levels (5.1, 5.0 and 5.1 g/dl, respectively) during the experimental period compared to the control group (4.7 g/ dl). Supplementation with Echinacea extract enhances the immune response of non-infected does in group B earlier than the infected groups (C and D).

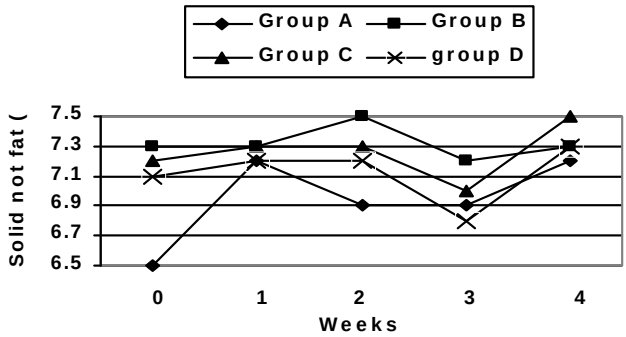


Figure 7: Solid not fat (%) in milk samples before treatment (0), during treatment (weeks 1, 2) and after the end of the treatment (weeks 3 and 4) in goat of groups A, B, C and D.

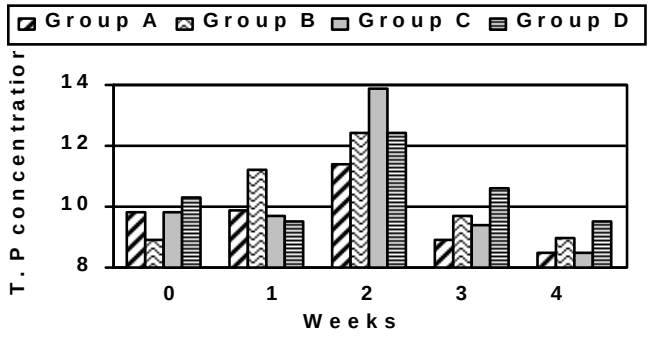


Figure 8: Total protein concentrations (g/ dl) in serum before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) in goat of groups A, B, C and D.

Table 5: Least square means (± SE) of serum total protein (STP, g/dl), albumin (Alb., g/ dl), globulin (glob, g/ dl) and α, β and γ-globulin (%) as affected by health status of the udder and treatment in Zaraibi goats.

Effect	STP g/ dl	Alb. g/ dl	Glob. g/ dl	α- glob. %	β- glob. %	γ- glob. %
HS:	NS		NS	NS		
Healthy	10.0± 0.3	5.1± 0.1 [a]	4.9± 0.3	10.3± 0.5	15.5± 0.5 [a]	19.6± 0.6 [a]
Infected	10.3± 0.3	5.3± 0.1 [b]	5.1± 0.3	10.7± 0.5	13.3± 0.5 [b]	22.5± 0.6 [b]
Treat (HS):	NS		NS	NS		
A	09.7± 0.4	5.0± 0.1[a]	4.7± 0.4	10.6± 0.8	15.5± 0.8 [a]	19.8± 0.9 [a]
B	10.3± 0.3	5.1± 0.1 [a]	5.1± 0.3	10.1± 0.8	15.5± 0.8 [a]	19.4± 0.9 [a]
C	10.2± 0.3	5.3± 0.1 [b]	5.0± 0.3	09.8± 0.8	14.5± 0.8 [a]	21.9± 0.9 [ab]
D	10.4± 0.4	5.3± 0.1 [b]	5.1± 0.4	11.6± 0.8	12.1± 0.8 [b]	23.0± 0.9 [b]

NS= non significant.

a, b, c, d= Means with the different letters are not significantly different.

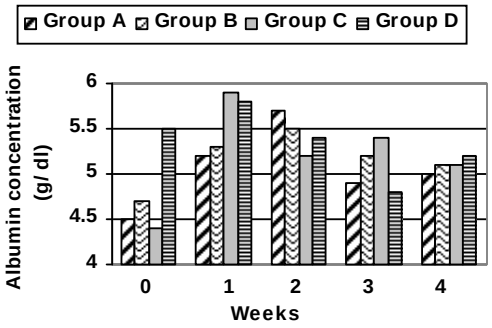


Figure 9: Albumin concentrations (g/ dl) in serum before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) of goat in groups A, B, C and D.

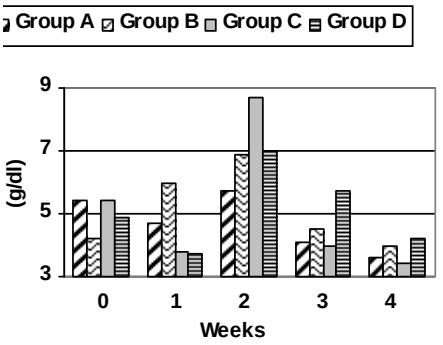


Figure10: Globulin concentrations (g/ dl) in serum before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) of goat in groups A, B, C and D.

Serum total protein and globulin levels start to increase at the first week of the treatment (Figure 8 and 10). However, serum total protein levels were significantly higher (11.4, 12.4, 13.9 and 12.4 g/ dl) in all groups (A, B, C and D) at the 2nd week during the treatment in comparison with its initial levels (9.8, 8.9, 9.8 and 10.3 g/ dl), respectively, where goat groups treated with Echinacea extract showed the highest levels (Figure 8). Globulin levels in serum samples in all groups followed the same trend of the total protein, where its level increased significantly at the 2nd week during the treatment (Figure 10). Globulin levels were significantly higher in goat groups that received Echinacea extract (6.9, 8.7 and 7.0 g/dl) for does in groups B, C and D, respectively than that in the control group A (5.7 g/dl).

groups received Echinacea extract (B, C and D) exhibited significantly higher serum γ-globulins (20.5, 21.7 and 23.7%, respectively) than that in the control group (18.6%) at the 2nd week during treatment (Figure 15). Changes of serum γ-globulin levels during the treatment were parallel to those of serum total protein and globulin levels, which reflects animal immune status and support the results that Echinacea extract stimulate goat's own defense system. It was noticeable that infected does in group D exhibited higher γ-globulin (Figure 15) and lower β- globulin levels (Figure 16) before the treatment (week 0) than that in group (C). The observed high γ-globulin level, which associated with a decrease in β-globulin levels, may be related to the high degree of infection in this group. This observation was supported by results of milk bacteriology and log SCC (Table 2 and Figure 1, respectively), where average counts of major bacterial types and log SCC in milk samples of does in group D were higher than that in group C.

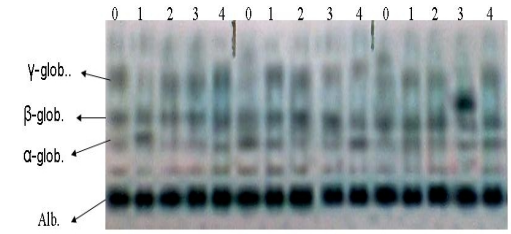


Figure 11: Electrophoretic identification of γ, β, α globulin and albumin in serum samples of 3 goats in group (A) before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) .

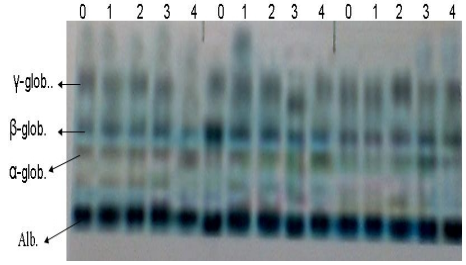


Figure 12: Electrophoretic identification of γ, β, α globulin and albumin in serum samples of 3 goats in group (B) before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4).

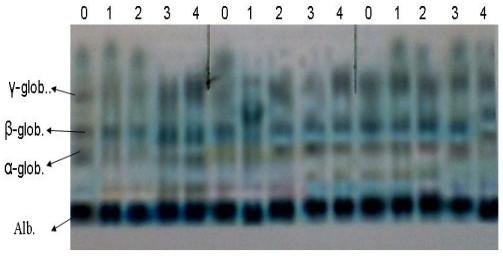


Figure 13: Electrophoretic identification of γ, β, α-globulin and albumin in serum samples of 3 goats in group (C) before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4).

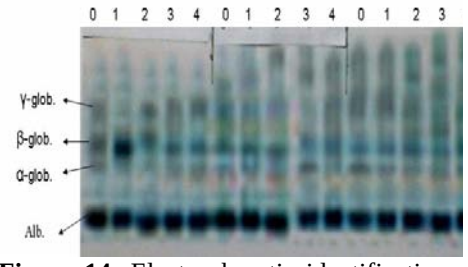


Figure 14: Electrophoretic identification of γ, β, α- globulin and albumin in serum samples of 3 goats in group (D) before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4).

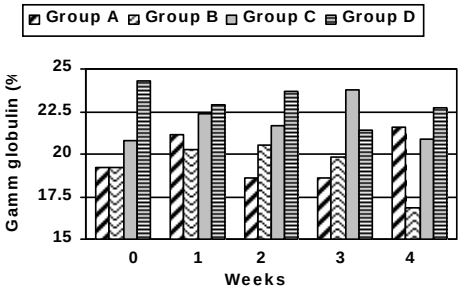


Figure 15: Percentage of γ-globulin concentrations from electrophoretic separation of serum samples before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) of goat in groups A, B, C and D.

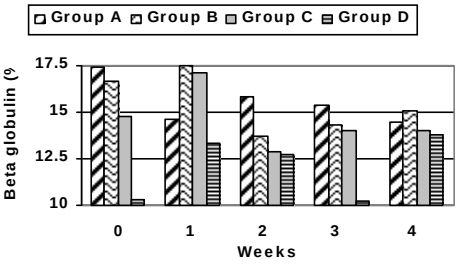


Figure 16: Percentage of β- globulin concentrations from electrophoretic separation of serum samples before treatment (0), during treatment (weeks 1, 2) and after treatment (weeks 3 and 4) of goat in groups A, B, C and D.

Results of the present study showed that using of Echinacea extract had a positive effect on milk SCC, milk yield and milk composition traits, as well as, serum total protein, globulin and γ-globulin levels. Moreover, no significant differences were detected between the two levels of the treatment by Echinacea extract (525 and 1050 mg/ day/ head). Therefore, the use of the low level of Echinacea extract is recommended in order to get better economic return.

CONCLUSION:

Data obtained in the present study showed a significant effect of supplementation with Echinacea extract on lowering SCC in milk of Zaraibi goats. It also, causes an increase in serum total protein, globulin and γ-globulin levels during the treatment. These results may prove its efficiency in activating goat's immune system. Moreover, Echinacea extract slightly improved both milk fat and protein percentages of the treated groups. Therefore, the use of Echinacea extract as immuno-stimulating additive is consider useful in improving health of the mammary gland, goat's immune response and improve health-promoting quality of milk. Since there were no significant differences between the two levels of Echinacea extract used (525 and 1050 mg/ day/ head) in this experiment on the studied traits, the lower level is therefore, recommended.

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