

Effect of Oral Administration of a Functional Synbiotic Syrup on Libido, Semen Characteristics, Serum Testosterone and Liver and Kidney Function of Goat's Bucks

¹K.A. Al-Sobayil, ¹M.M. Zeitoun, ¹M.H. Khalil and ²A.M. Abdel-Salam

¹Department of Animal Production and Breeding

²Department of Food Science and Human Nutrition,

College of Agriculture and Veterinary Medicine,

Qassim University, Buraydah, Al-Qassim, Saudi Arabia

Abstract: The present study aimed at investigating the biological evaluation of a synbiotic fermented milk synergistic with some active ingredients of herbal hydrosols and honey on the sexual activity, semen characteristics and testosterone levels in Aradhi and Damascus goat's bucks. A synbiotic syrup was prepared by mixing fermented cow's milk containing some probiotic strains and natural antioxidants isolated from functional food and herbal hydrosols. Fifteen Damascus and Aradhi bucks were utilized in the experiment. Two bucks served as control (given no synbiotic), 7 bucks were designed to be orally given twenty ml of the mixture three times per week for 8 consecutive weeks (Low dose, Aradhi-Low = AL, Damascus-Low = DL) and 6 bucks were orally given 40 mL of the mixture (High dose, Aradhi-High = AH, Damascus-High = DH) by the same regime. Bucks were tested for the libido and semen ejaculates were collected once a week for testing the physical characteristics. A jugular blood sample was taken once a week in a non-heparinized tube for the determinations of serum testosterone, alanine and aspartate amino transaminases (ALT and AST), urea and creatinine levels. Results indicated a significant enhancement of the libido due to the breed of the buck; Aradhi bucks exhibited less reaction time than Damascus bucks ($p < 0.01$). The high dose of the mixture enhanced ($p < 0.01$) the libido in both breeds. Testosterone levels were significantly ($p < 0.01$) higher in Aradhi than Damascus bucks. There found significant increase in testosterone concentrations in treated Aradhi than in Damascus bucks. There were no differences between low and high mixture dose. Administration of the mixture resulted in an increased ($p < 0.01$) ejaculate volume, gross and individual motility, sperm concentration, total motile sperm in the ejaculate than in control. Additionally, lower dead and abnormal sperm numbers were obtained with bucks given the mixture. Treatment slightly increased AST and urea but significantly increased ALT and creatinine in both breeds. In conclusion, giving goat bucks a mixture of a synbiotic functional syrup enhanced the metabolic activity resulting in improvements in their reproductive performance.

Key words: Synbiotic fermented milk, herbs, hydrosols, honey, sexual activity, goat semen, testosterone

INTRODUCTION

Complementary medicine has gained an increased popularity in the recent years. Food manipulation and traditional plant therapies are commonly prescribed by ayurvedic and other folk systems of medicine in various countries including Middle East. Oral consumption of probiotics

(live microbial feed supplement that enhance the host health by modulating the intestinal microbial balance) has been associated with the prevention, alleviation or cure of diverse intestinal disorders such as lactose intolerance, viral and bacterial diseases (McNaught and MacFie, 2001).

Milk contains components that provide critical nutritive elements, immunological protection and biologically active substances to both neonates and adults. In general, the major protein fractions in bovine milk include α -lactalbumin, β -lactoglobulin, caseins, immunoglobulin, lactoferrin, proteose-peptide fractions (heat-stable, acid soluble phosphoglycoproteins) and minor whey proteins such as transferrin and serum albumin. From these, bioactive peptides may be generated in vivo through gastrointestinal processes (Clare and Swaisgood, 2000).

Probiotic may also be a functional food, but more specifically it is a live microbial feed supplement that beneficially affects the host beyond correcting for traditional nutrient deficiencies by improving its intestinal balance (Fuller, 1999). Hence, it may be considered a functional food with the special property of containing live, beneficial microorganisms (McIntosh, 1996). A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson, 2004).

Probiotics, prebiotics and synbiotics aimed at improving intestinal health currently represent the largest segment of the functional foods market. Evidence continues to emerge demonstrating that these ingredients have a potential to improve human health in specific intestinal disorders (Fabian and Elmadfa, 2006).

Hydrosols, also known as floral water, distillate water or aromatic water, are the co-products or the by-products of hydro and steam-distilled plant materials. Hydrosols are quite complex mixtures containing traces of the essential oils and, of course, several water-soluble components. They have practically been used as beverages for a long time in the middle eastern countries. Some of herbs have commonly been used in foods mainly for their flavor, aromas and preservabilities. Additionally, herbal tea have been used as alternative medicines and natural therapies two decades ago. hydrosols, widely used in food products and as drinks, were tested for their inhibitory effects against pathogenic bacteria such as *E. coli* O157:H7, *Staphylococcus aureus* and *Yersinia enterocolitica*, it is likely that some edible plant hydrosols may be used as antimicrobial agents to prevent the deterioration of food products (Sağdıç, 2003; Sağdıç and Özcan, 2003).

The recent advances in such a studies have promoted us to investigate the effect of such a mixture on the male sexual behavior and semen quality. Therefore, the objective of the present study aimed at investigating the effect of oral administration of a safely nutritive mixture on the libido, semen characteristics, male sex hormone (Testosterone) and kidney and liver functions of goat bucks raised under hot climate.

MATERIALS AND METHODS

Probiotic Fermented Milk Cultures

Starter cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen's Laboratory, Copenhagen Denmark.

Preparation of Probiotic Fermented Milk

Probiotic fermented milk was prepared according the method described by Tamime and Robinson (1999) and Al-Wabel *et al.* (2007). Probiotic cultures (*Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) were inoculated and incubated for 4-8 h at 42°C. After coagulation, the curd were tested for pH, then was stirred in an electric blender and stored refrigerated (4-6°C). Chemical compositions analysis were carried out according to the official method (AOAC, 2000).

Preparation of Medicinal Plant Hydrosols

Hydrosols of the used medicinal plants were prepared by the hydro-distillation according the method described by Deans and Svoboda (1990) and Bakri and Douglas (2005). About 100 g of plant materials were cut into small pieces, placed in a flask (2 L) with 1000 mL of double distilled water and hydro-distilled for 1 h. After hydro-distillation, the mixture with trace essential oils in the flask was identified as hydrosol. The amount of obtained hydrosol was approximately 200 mL. The hydrosol was then filtered and preserved in sterile dark bottles (500 mL) in a cool environment (4°C) until further use.

Preparation of Synbiotic Fermented Milk

Synbiotic fermented milk was prepared by adding the active ingredients of herbal hydrosols to the probiotic fermented milk at a 3% (30 g L⁻¹), apple acid at 1% (10 ml L⁻¹) and honey at 30% (300 g L⁻¹). Chemical composition and sensory evaluation for the appearance, color, flavor and overall properties of probiotic and synbiotic fermented milk were adopted from NASA (1999): very good, (++) good, (+) accepted and (-) unaccepted.

Table 1 and 2 show the chemical composition of probiotic fermented milk and honey.

Animals

Fifteen adult Damascus (n = 11) and Aradhi (n = 4) goat bucks were individually housed in a closed pen. Each animal was offered 300 g concentrated pelleted ration (13% crude protein) in addition to an Alfa Alfa hay and water as a free choice. Seven bucks were orally given 20 mL of the synbiotic mixture every other day (3 times/week) for a period of 8 weeks (Low-dose group). Six bucks were given 40 mL of the same mixture on the same schedule (High-dose group). Two bucks served as control (given no synbiotic). Buck's libido was estimated by the time (seconds) lapsed from releasing the buck outside its pen until the penis was completely erected upon riding the estrous doe.

Semen Collection

Since these bucks are designed as sires for semen collection to inseminate goat does in a research project, there has been a record for their semen traits before application of the synbiotic material. At the commencement of the experiment, semen ejaculates were collected just before administering the material and just after administration of the material thereafter. An estrous doe was brought to the male location and the buck was released from its pen and using a stop watch, the time (sec) elapsed from its release until the complete penile erection was recorded as an indicator of the libido. A regular artificial vagina for sheep and goats was used.

Semen Parameters

The ejaculate volume (mL), semen pH, gross motility, individual progressive motility, percent dead sperm, percent first and second sperm abnormalities, sperm concentration (Neubaur haemocytometer), estimated Total Sperm Output (TSO) and estimated Total Motile Sperm (TMS) in the ejaculate. The TSO and TMS were estimated as follow:

Table 1: Chemical composition of probiotic fermented milk (%)

Solids not fat	Carbohydrate	Fat	Protein	Ash
8.81	4.66	3.20	3.0	0.90

Table 2: Chemical composition of honey

Carbohydrate	Water	Fructose	Glucose	Galactose
82.12	17.10	35.75	35.75	3.10

TSO = Ejaculate volume (mL) × Sperm concentration (No. mL⁻¹)

TMS = TSO × Percent progressive motility

Blood Sampling and Sera Harvesting

A venipuncture procedure was used to withdraw blood samples of the jugular vein once per week of the bucks just before semen collection. Blood was collected in non-heparinized vacutainer tubes. Blood samples were cooled in the refrigerator (4-6°C) for two hours and centrifuged at 3000 x g for 30 min. Sera were harvested, labeled and stored deep frozen (-70°C) until used for assays.

Testosterone Determinations

Testosterone was determined in serum by the EIA method (Rassaie *et al.*, 1992; Raji *et al.*, 2005) using the horse-raddish peroxidase as a tracer and Tetra Methyl Benzidine (TMB) as a chromogen (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). The intra and interassay coefficient of variation were 5.2 and 7.6%, respectively.

ALT and AST, Urea and Creatinine Analyses

Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST) activities were determined according to the method of Reitman and Frankel (1957).

Urea and creatinine were determined in serum according to the methods of Tietz (1970) and Bonsnes and Taussky (1945), respectively.

Statistical Analysis

Data of testosterone, blood metabolites and semen physical characteristics as repeated measures on the same buck were analyzed using GLM procedure of SAS program (SAS, 1996). The following linear model was applied:

$$Y_{ijkl} = \mu + T_i + B_j + W_k + e_{ijkl}$$

Where:

Y_{ijkl} = Observation on $ijkl^{\text{th}}$ ejaculate

μ = Overall mean

T_i = Fixed effect of i^{th} treatment (breed-dose).

B_j = Random effect of j^{th} buck within i^{th} treatment

W_k = Fixed effect of k^{th} week of sampling

e_{ijkl} = Random error

RESULTS AND DISCUSSION

The sensory evaluation properties were found to have good scores and were acceptable for consumers (Table 3).

Effect of Synbiotic on Buck's Libido

Administration of the synbiotic mixture caused significant ($p < 0.01$) stimulation for buck's sex drive, this was shown in the reduction of the duration (seconds) between buck release from its pen until a complete erection was achieved. Damascus bucks given the Low Dose (DL) were the only exception since they didn't show an improvement in their libido as compared to the controls. The duration per seconds for the bucks were; 27.4 ± 2.17 , 29.31 ± 1.36 , 12.01 ± 1.36 , 17.37 ± 1.7 and

Table 3: Sensory evaluation properties of functional probiotic and synbiotic fermented milk

Sensory evaluation	Probiotic fermented milk	Synbiotic fermented milk
Appearance	+++	+++
Color	+++	++
Flavor	+++	++
Overall	+++	+++

+++; Very good, ++; Good, +; Accepted, -; Un-accepted

Table 4: Effect of treatment with synbiotic on serum testosterone and semen characteristics of Damascus and Aradhi goat's bucks (LSM±SEM)

Trait	Treatment ^o				
	Control	DL	DH	AL	AH
Testosterone (ng mL ⁻¹)	0.95±0.67 ^a	0.77±0.44 ^b	1.06±0.49 ^b	6.33±0.64 ^c	5.92±0.64 ^c
Ejaculate vol. (mL)	1.46±0.11 ^d	1.36±0.07 ^c	1.81±0.06 ^c	1.86±0.09 ^c	2.46±0.09 ^c
Gross motility score	2.94±0.16 ^f	3.49±0.08 ^g	3.94±0.07 ^g	3.69±0.10 ^f	4.00±0.10 ^f
Individual motility (%)	58.06±3.04 ^d	71.02±1.48 ^e	80.20±1.29 ^h	75.94±1.8 ^e	81.77±1.8 ^e
Sperm conc. (×10 ⁹ mL ⁻¹)	1.22±0.14 ^d	2.11±0.09 ^e	2.68±0.08 ^e	2.71±0.11 ^b	3.06±0.11 ^b
TSP ^a (×10 ⁹ mL ⁻¹)	2.30±0.49 ^d	3.70±0.31 ^e	5.35±0.27 ^e	5.58±0.38 ^e	7.78±0.37 ^e
TMS ^{**} (×10 ⁹ mL ⁻¹)	1.46±0.56 ^d	2.90±0.27 ^e	4.55±0.24 ^e	4.48±0.34 ^e	6.60±0.33 ^e
Dead sperm (%)	44.72±2.53 ^a	24.90±1.6 ^b	15.55±1.6 ^c	16.19±1.99 ^c	14.50±1.93 ^c
Abnormal sperm (%)	16.99±1.27 ^a	15.10±0.8 ^b	8.93±0.8 ^c	8.19±1.0 ^c	9.37±0.97 ^c

^aTSP: Total Sperm Output/ejaculate, ^{**}TMS: Total Motile Sperm/ejaculate, Means within a row with different superscript significantly differ at p<0.01, ^oTotal number of ejaculates in the experiment were 120 (8 ejac./animal)

13.99±1.65 for control, Damascus-Low dose (DL), Damascus-High dose (DH), Aradhi-Low dose (AL) and Aradhi-High dose (AH), respectively. Libido was also improved by time as frequent doses of the mixture were given.

Effect of Synbiotic on Serum Testosterone and Semen Characteristics

As shown in Table 4 there found significant (p<0.01) increases in testosterone concentration in the Aradhi bucks. However, no significant (p>0.05) differences were found in testosterone concentrations in Damascus bucks when compared to the control. Therefore, breed difference was found as mean levels of testosterone in Aradhi bucks approached 6 folds that found in Damascus ones. Dose of the synbiotic mixture did not show difference in either breed. Values of pH ranged between 6.8-7.1 and were not affected by breed, treatment dose or week of sampling.

There also found significant (p<0.01) increases in ejaculate volume of treated bucks as compared with control. Increasing the dose of the mixture resulted in significant ejaculate volume increase within breed. Mean ejaculate volume was higher in Aradhi (2.16 mL) than in Damascus (1.58 mL) bucks.

Gross as well as individual progressive motility were improved (p<0.01) by the administration of the mixture. Increasing the mixture dose caused significant increases of motility parameters within breed. On the contrary, there was no significant difference in percentage of progressive motility among breeds. The mean values of progressive motility were; 58.06, 71.02, 80.20, 75.94 and 81.77% for control, DL, DH, AL and AH, respectively.

There also found a significant (p<0.05) increase in sperm concentration in treated than control bucks. Moreover, increasing the dose of the mixture significantly increased sperm concentration within breed. The values of sperm concentration were; 1.22±0.14, 2.11±0.09, 2.68±0.08, 2.71±0.11 and 3.06±0.11×10⁹ sperm mL⁻¹ for control, DL, DH, AL and AH, respectively. Likewise, data for the estimate of total sperm output/ejaculate and total motile sperm/ejaculate exhibited similar trends. The values of total sperm output were; 2.30±0.49, 3.70±0.31, 5.35±0.27, 5.58±0.38 and 7.78±0.37×10⁹ for control, DL, DH, AL and AH, respectively. The respective values for total motile sperm/ejaculate were ;1.46±0.56, 2.90±0.27, 4.55±0.24, 4.48±0.34 and 6.60±0.33×10⁹ sperm. Contrariwise, there existed a significant (p<0.01) decrease in percentage of dead and abnormal sperm in treated as compared with control bucks. The treatment reduced percentage of dead sperm to reach 30% of these values obtained with control bucks (Table 4).

Many studies have shown that probiotics can alleviate lactose intolerance, lower serum cholesterol, reduce diarrheal incidence, stimulate the immune system, control infections, act as antibiotics, suppress tumors and protect against colon/bladder cancer by maintaining a healthy intestinal micro flora balance (Brigidim *et al.*, 2001; Rolfe, 2000; Oberhelma and Alvarez-Olmos, 2001; Fuller, 1999; Lee and Salminen, 1995). These probiotics have shown several mechanisms in the live mammalian body. Such mechanisms include production of antibacterial substances, competition for nutrients and adhesion sites in the lower gut and stimulation of host defense mechanisms. There also found a release of antibacterial peptides from Paneth cells and an increase in brush border enzyme activity (Buts *et al.*, 1986; Satoh, 1988). Moreover, these probiotics modify the host immune responses which appeared to be mainly enhanced by activating macrophage functions and increasing the activity of normal killer cells and T-cells (Kato *et al.*, 1994).

The ingredients of probiotics are mainly Lactic acid bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) which proved to enhance the functions of the lower gut (Rafter, 2003). Additionally, there might have a synergistic effect between these bacteria and rumen microorganisms to enhance their functions in rumen absorption. Also, some lactic acid bacteria have been shown to increase colonic NADPH-cytochrome P-450 reductase activity (Pool-Zobel, 2005) and glutathione S-transferase levels (Challa *et al.*, 1997). Obviously, these enzymes which are involved in several metabolic processes including spermatogenesis and steroidogenesis might enhanced such physiological functions in the treated bucks.

Furthermore, the inclusion of active herbal ingredients (i.e., fructo-oligosaccharides (FOS), terpenoids, saponins, lactoferrin, 4-hydroxyl leucine folate, tannins, phosphoproteins, alkaloids, panaxoside and dietary fibers) in addition to honey ingredients in such a mixture could have some stimulatory effects on the testes level. Panaxoside is an active ingredient of the Korean Ginseng which have been long known as an aphrodisiac herb.

Table 5 shows data of concentrations of serum urea and creatinine as bio indicators of renal function and data of activities of aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) as bio indicators of liver function. There were no significant changes in levels of AST and urea in serum of treated than control bucks. Contrariwise, there found a significant ($p < 0.01$) elevation in ALT and creatinine only in treated Aradhi bucks. Apparently, there were no significant differences due to mixture dose. Since urea levels in serum is commonly used as a bio-indicator for renal function, in the current study there found no changes in urea levels due to treatment which indicates that administering this mixture didn't affect protein metabolism. The increase in creatinine levels were only encountered in Aradhi bucks which might be ascribed to the genetic-makeup of the breed.

Additionally, Johnston (1999) indicated that there are other factors, rather than liver or kidney function, cause several times increase than normal levels of creatinine, AST and ALT. Of these factors were muscular exercise and obesity. These bucks were left free in the open yards with does for natural mating. Interestingly, the Aradhi have extra movement activities than Damascus bucks. It has been also indicated (Johnston, 1999) that slight elevations of AST and ALT (within 1.5 times the upper limits of normal values) do not necessarily indicate a liver dysfunction. Moreover, Johnston (1999) found differences in blood plasma ALT levels due to different genetic origins. Enzymes, generally, are protein catalysts present mostly in living cells and are constantly and rapidly degraded although regenerated

Table 5: Effect of oral administration of a synbiotic mixture on liver and kidney function of Damascus and Aradhi goat bucks (LSM \pm SEM)

Trait	Control	DL	DH	AL	AH
AST (U L ⁻¹)	103.45 \pm 12.93 ^a	121.86 \pm 12.93 ^{ab}	144.20 \pm 14.78 ^a	118.14 \pm 12.93 ^{ab}	118.71 \pm 12.93 ^{ab}
ALT (U L ⁻¹)	18.36 \pm 1.96 ^a	21.63 \pm 1.96 ^{ac}	19.75 \pm 2.26 ^a	26.03 \pm 1.96 ^b	27.84 \pm 1.96 ^b
Urea (mg dL ⁻¹)	42.46 \pm 3.25 ^{ab}	34.39 \pm 2.90 ^b	23.17 \pm 2.75 ^b	41.30 \pm 2.90 ^b	48.41 \pm 3.11 ^a
Creatinine (mg dL ⁻¹)	0.94 \pm 0.09 ^a	0.94 \pm 0.06 ^b	0.99 \pm 0.06 ^b	1.18 \pm 0.08 ^a	1.31 \pm 0.08 ^a

^aMean in the same row with different superscript significantly differ ($p < 0.01$)

by new synthesis (Coles, 1986). According to Zilva and Panall (1984), normal enzyme level in blood serum is a reflection of a balance between its synthesis and release, as a result of different physiological processes in the body. Transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the process known as transamination (Carola *et al.*, 1990).

Although, no previous studies have been done to test effects of probiotics on ruminants, there must taken into account the differences in metabolic processes between monogastric and ruminants. Because the oral administration of such a mixture to goat's bucks must be encountered by the populations and activities of various types of rumen microorganisms, we can't extrapolate, at this point the results derived of the mice or human studies to be applied on ruminants. The sources of energy, enzymes, minerals and vitamins could be utilized as useful constituents not only for the animal body, but also for the metabolic processes of rumen microorganisms.

In conclusion, oral administration of a symbiotic mixture containing functional food constituents to goat males enhanced their libido and semen physical characteristics. Although, much research needs to be done on the effect of each strain of *Lactobacillus*, each constituent of the fermented milk, herbs and honey on the ruminant reproductive physiology. This must be carried out on the levels of rumen ecology as well as on the reproductive organs and subsequent fertility.

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