Journal of Cell and Animal Biology

Volume 8 Number 1 January 2014

ISSN 1996-0867

Academic Journals

ABOUT JCAB

The **Journal of Cell and Animal Biology (JCAB)** (ISSN 1996-0824) is published Monthly (one volume per year) by Academic Journals.

Journal of Cell and Animal Biology (JCAB) provides rapid publication (monthly) of articles in all areas of cell and animal biology such as Cellular metabolism, Cellular differentiation, Alcoholic fermentation etc. All articles published in JCAB are peer-reviewed.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email jcab@academicjournals.org.

With questions or concerns, please contact the Editorial Office at jcab@academicjournals.org.

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.
Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Cell and Animal Biology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Cell and Animal Biology

Table of Contents: Volume 8 Number 1 January, 2014

ARTICLES

academicJournals

Vol. 8(1), pp. 1-14, January 2014
DOI: 10.5897/JCAB12.043
ISSN 1996-0867 ©2014 Academic Journals
http://www.academicjournals.org/JCAB

Journal of Cell and Animal Biology

Review

Physiological responses of dairy animals to recombinant bovine somatotropin: A review

E. B. Soliman and M. A. A. EL-Barody*

Animal Production Department, Faculty of Agriculture, Minia University, Minia, Egypt.

Accepted 2 December, 2013

Recombinant bovine somatotropins (rbST) have been synthesized and manufactured using recombinant DNA (rDNA) techniques. The rbST has increased milk production in dairy animals, including cow, sheep and goats. There are management factors including dosage of rbST, injection interval, genetic potential and environmental conditions which affect the magnitude of the animal-response to rbST. For dairy cows, the optimal dose of rbST treatment is between 25 and 50 mg/day. Daily injection of rbST may produce better response, but sustained-release formulations of rbST are more practical. The magnitude of milk production response to rbST range from 10 to 35% in dairy cows. The rbST has no or little effects on the milk composition, processing properties and taste. The rbST did not affect digestion of feeds or the efficiency of utilizing absorbed nutrients for milk synthesis, and it has no or little effect on rumen fermentation, flow of nutrients to the small intestine and total tract digestibility, but it improves the feed efficiency in dairy cows. The rbST induced significant increase in serum bST. It has a galactopoietic effect, since it increase the metabolic activity of mammary cells or slow their involution, thereby allowing more secretory cells to persist over time and contribute to increasing milk synthesis and yield. It has a great impact on mammary gland development and subsequently milk-producing capacity in dairy heifers. The rbST increase the hypothyroid status of lactating cows and maintain euthyroid condition in the mammry gland, thus enhancing its metabloic priority. The rbST did not adversely affect reproduction, and the observed decreases in reproductive performance in rbST-treated cows may be attributed more to the increases in milk yield than to direct effect of rbST. The increased production responses to rbST in heat-stressed dairy cows is less than that under more moderate conditions. The incidence of mastitis in rbST-treated cows is due more to increased milk yield than to any direct effects of rbST. The rbST was efficacious in increasing milk yields in sheep and goats without adverse effects.

Key words: Recombinant bovine somatotropin, dairy animals, physiological responses.

INTRODUCTION

Bovine somatotropin (bST) is naturally-occuring protein produced by the pituitary gland in all cattle. Recombinant bovine somatotropins (rbST), which differ from their native form by several amino acids, have been synthesized and manufactured using recombinant DNA techniques to increase milk production in dairy cows. The Food and Drug Administration (FDA) approved rbST product in 1993 after determining that its use would be safe and effective. Part of FDA's safety evaluation was to ensure that milk from treated cows was safe for human food. U.S. commercial use began in 1994, and adoption has been extensive. The safety of rbST was evaluated by the American Medical Association, American Academy of Pediatrics, American Cancer Society, Council on Agricultural Science and Technology, Food and Nutrition Science Alliance, Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Without exception, all of the

*Corresponding author. E-mail:profelbarody@yahoo.com.

medical associations and scientific societies concluded that use of rbST represented no health or safety concerns for consumers or cows. Biomarker-based screening for rbST can be considered a very promising start for detecting rbST abuse in dairy cows. Development of a flow cytometric immunoassay for rbST-induced antibodies in serum of dairy cows is considered as an important biomarker for pinpointing at rbST abuse in cattle (Smits et al., 2012). In addition, the osteocalcin-anti-rbST antibodies combination represents fit-for-purpose biomarkers for screening of rbST abuse in dairy cattle. This screening method can be incorporated in routine veterinary monitoring programmes in the European Union for detection of rbST abuse and in the control of rbST-free dairy farms in the United States of America and other countries (Ludwig et al., 2012). The hormonal induction of lactation in barren high-yielding cows is a reliable, practical and affordable technique in countries where rbST treatment of dairy cows is legally permitted (Mellado et al., 2011). Use of rbST in dairy cows is an excellent example of the development and integration of basic research derived from several fields into a new management practice that can influence the efficiency and profitability of milk production without adversely affecting milk quality or animal health (Phillips, 1996), and have less negative effects on the environment than conventional dairying (Capper et al., 2008). Most research has been with Holsteins, but milk production responses have been reported for all dairy breeds examined including North American and Eurpean breeds (Bauman, 1992), South American breeds (Mattos et al., 1989) and African breeds (Madakaze et al., 1990). In this review article, the mechanistic aspects involved in the physiological responses of dairy animals to rbST in relation to their productive and reproductive performances was focused on and discussed.

EFFECT OF rbST ON LACTATING COWS

Effect of rbST on milk production

Use of rbST treatments has increased milk production in all dairy breeds examined, including *Bos indicus* cows (Phipps et al., 1991). Most of studies were conducted for one lactation or less, however, an important consideration for the commercial application of rbST is the milk yield response over multiple lactations (Phillips, 1996). So, many studies have evaluated the response to rbST for 2 or more lactations. The rbST-induced increases in milk yield were reported to be maintained for two consecutive lactations (Phipps et al., 1990) or over four lactations (Huber et al., 1991). Management factors have been identified as major source of variation in the magnitude of dairy cows responses to rbST (Bauman, 1992). These factors include dosage of rbST, injection interval, genetic potential and environmental conditions. According to Phillips (1996), cows that are better managed are known to have a greater response to rbST than poorly managed, and producers that manage their operation to maximize milk production have the greatest potential to maximize milk yield response to rbST. For dairy cows, the optimal dose of rbST treatment as a galactopoietic agent is between 25 and 50 mg/day (Downer et al., 1993; Phillips, 1996). Given an adequate dosage, increasing the milk yield in response to rbST was maintained by following the rbST administration daily and every 7, 14 or 28 days (Zinn et al., 1993; Chalupa et al., 1996). Low doses of rbST (10.2 mg/day) in the transition period resulted in higher postpartum body weight, quicker recovery of body condition during lactation, and significantly more milk during treatment (Gulay et al., 2003).

The magnitude of milk yield response to rbST were reported to be increased by 7, 19, 21 and 24% with 5, 10, 15 and 20 mg/day (West et al., 1990); 7 and 9% with 10.3 and 25 mg/14 days (Zhao et al., 1992); 9, 14 and 12% with 11.4, 22.8 and 22.9/28 days (Laurent et al., 1992) 0, 12 and 25% with 7.1, 14.3 and 21.4 mg/7 days (Zinn et al., 1993), 18% with 250 mg/14 days (Ocampo et al., 1995), 12.2 and 20.0% with 250 and 500 mg/14 days (Abdel-Rahman et al., 2010) and 22% with 500 mg/14 days (Thammacharoen et al., 2011).

Although, rbST daily injection may produce better response (Bauman, 1992), administration of sustained-release formulations of rbST are more practical (Fernandez et al., 1995). However, the increase in milk yield with sustained-release formulations of rbST within a single injection interval will vary (Zinn et al., 1993). That is, following each injection, the milk yield will increase to a peak, approximately at the mid-point of the injection interval, and then decline until the next injection (Phillips, 1996).

Some studies indicated that the rbST-induced milk production was accompanied by altering plasma factors. For example, Gallo and Bloke (1990) working on multiparous cows receiving rbST 350 mg/14 days from 98 to 305 days postpartum, found that milk yield was increased by 20.4 and 3.5% FCM yield by 16.7%. Also, rbST decreased blood pH and buffer capacity by decreasing bicarbonate without affecting blood pressures of O_2 and CO_2. Plasma albumin was decreased while plasma glucose, insulin and non-etherified fatty acids (NEFA), were increased with rbST treatment at 500 mg/14 days (Flores et al., 2007). However, Abdel-Rahman et al. (2010) found a non-significant increase in serum insulin accompanied by a remarkable drop in serum glucose in primiparous cows that received 250 and 500 mg rbST and multiparous cows injected with 500 mg rbST/14 days. A possible explanation of such hyperinsulinemia accompanied by hypoglycemia is claimed by Gong et al. (1997) who stated that the action of bST may be mediated by increase synthesis and secretion of IGF-1 and insulin. They also showed no significant change in the activity of aspartate aminotrans-

ferase (AST) and alanine aminotransferase (ALT) and the level of creatinine in all rbST-treated groups. The activities of serum ALT and AST which are commonly used as indicator of liver cell damage and death were not affected by treatment with rbST. Plasma total protein, cortisol, total lipids, triacylglycerol contents, AST, hemoglobin and packed cell volume were not affected by rbST, but the percentage of triacylglycerol in total lipids was increased with rbST. However, serum total lipids, triglycerides and cholesterol showed a non significant increase in multiparous cows given 500 mg rbST/14 days (Abdel-Rahman et al., 2010). It was claimed that somatotropin mobilizes large quantities of free fatty acids from adipose tissues to be used to supply most of the energy of the body, thus somatotropin acts as a potent carbohydrate and protein sparer (Kannan, 1987). Gallo and Bloke (1990) suggested that rbST had lipolytic and diabetogenic activities and those effects may be important for the increase in nutrient partitioning towards the mammary glands elicited by rbST.

As far as stage of lactation is concerned, studies that compared the effect of rbST at various periods of lactation showed that milk production responses to rbST is proportionately greater later in lactation than earlier (prior to day 60) (Phillips, 1996). In addition, Chalupa et al. (1996), working on dairy cows during weeks 5 through 43 of lactation, showed that the FCM production increased ($P<0.05$) with 10.3, 20.6 or 41.2 mg/day during week 4 to 36 of rbST treatment. During the final 4 weeks of lactation, 10.3 and 41.2 mg/day of rbST, but not 20.6 mg/day, promoted increase in FCM production ($P<0.05$). Administration of rbST prior to peak production in cows that are well fed, prevents the number of mammary cells from decreasing, and increases the amount of nutrients directed away from fat and toward the mammary cells, leading to an extension of peak milk production (Bauman, 1999).

Since there is a correlation between blood bST concentrations and genetic potential for milk production and/or actual milk production (Kazmar et al., 1990), there may be differences in rbST-induced milk production in cows of different genetic merit (Phillip, 1996). Cows with greater genetic merit may have a greater potential to respond to rbST (Gibson et al., 1992). However, in a study on dairy cows from 2 genetic groups (mated for 20 years to bulls of high or low transmitting ability for milk yield) and given 0, 10.3, 20.6 or 30.9 mg/day rbST from 14^{th} week after parturition for 30 weeks, the increase in milk, fat and protein yields due to rbST did not differ between genetic groups. Higher, doses of rbST increased milk yield, 4% FCM in a linear fashion (Nytes et al., 1990).

Effect of rbST on milk composition

Several investigators worked on dairy cows and showed that overall milk composition was not affected by rbST treatments at 100-780 mg/14 days (Burchard et al., 1990), 29 mg/day (Binelli et al., 1995) or 10.3-41.2 mg/day (Chalupa et al., 1996). Also, Phipps et al. (1996) showed no effect of prolonged-release formulation of rbST on milk composition of Holstein cows. Additionally, in early lactating cows that received rbST for 90 days, milk composition was not affected by rbST treatment (Santos et al., 1999). According to Chalupa et al. (1996), the unaffected milk fat, protein and total solids to rbST reflected the high nutrient densities of the diets, because effects of rbST on milk composition seemed to be related to nutritional status.

The rbST did not affect milk composition when cows were fed diets containing positive balances of energy and protein (Bauman, 1992). In general, effects of dietary energy and protein on milk fat and protein from rbST-treated cows were similar to alterations when high producing cows are fed diets that do not provide adequate energy or protein (Chalupa et al., 1996). On the other hand, many studies indicated variable responses in milk composition to rbST treatments. In dairy cows that received rbST at 640 mg/28 days, yields of fat, protein and lactose increased by 21, 18 and 16%, respectively, but there were no significant changes in protein, lactose, Ca, Mg and P in milk (Oldenbroek and Garssen, 1988).

In addition, cows injected with 30 mg of bST/day increased milk fat and lactose concentrations, but protein and fatty acid composition of milk were not affected (French et al., 1990). Also, McGuire et al. (1992) showed an increase in milk fat and total solids due to rbST at dose of 25 mg/day. But, percentage milk fat was not affected by rbST at 10.3 mg/day, while percentage protein was reduced (Austin et al., 1990). Response of milk composition in 10.3, 20.6 or 30.9 mg rbST-treated cows with different genetic merit indicated that yields of milk fat and protein were increased, but fat and protein percentages were unaffected (Nytes et al., 1990).

The increase in milk fat due to rbST treatment could be attributed to the increase in long chain fatty acids from body reserves mobilized when dairy animals were in negative energy balance (McDowell et al., 1988). The reduction in milk protein content with rbST dosages could be due to the increase in milk yields (Fernandez et al., 1995). Given little change in milk composition together with an increase in milk yield, yield of milk components was increased in the same proportion as the yield (Barbano et al., 1992). Evidences indicate that use of rbST in dairy animals has no or little effects on the milk composition, processing properties and taste (Phillips, 1996).

Effects of rbST on feed intake, nutrients digestibility, body weight, milk production efficiency and energy balance

Productive and nutritional performances of dairy cows have been monitored in milk production studies with

rbST. Some studies showed no change in feed intakes (FI) of dairy cows in response to rbST at 56-700 mg/14 days (Downer et al., 1993) or at 29 mg/day (Binelli et al., 1995); thereby resulting in increased feed efficiency (FE). Also, Windsryg et al. (1991) working on ruminally fistulated cows at 60 days postpartum injected with 25 mg of rbST/day for 6 weeks, showed that 3.5% FCM and milk production efficiency were increased with rbST treatment while DM intake, ruminal percentages of crude protein (CP), alpha-amino-N, volatile fatty acids (VFA), pH, total tract apparent digestibility of nutrients and cellulolytic bacteria (%) were not affected by rbST, but the total number of rumen protozoa tended to be higher with rbST. However, Santos et al. (1999) observed that early lactating cows that received rbST for 90 days had lower dry matter intake (DMI) during the first 45 days of experiment, but efficiency of feed utilization was increased with rbST while nutrients digestibility was unaffected. Similar observations were reported by Schwab and Kirchgessner (1990) who found that apparent digestibilities of DM, CP, crude fibers (CF), nitrogen-free extract and energy were not altered in dairy cows that received rbST at 640 mg / 28 days for 168 days. Also, efficiency of milk production was improved in cows treated with rbST at 640 or 960 mg /28 days during 3 successive lactations (Oldenbroek and Garssen, 1990). Since, the above-metioned responses to rbST coincided with increased milk production, extra nutrients required for this increased milk yield are thought to come from mobilization of body tissues (Phillips, 1996).

On the other hand, an increase in DMI by 46% due to rbST (500 mg /14 days), and the commencement of the DMI decrease was correlated with the beginning of the decrease in milk production (Moallem et al., 2000). It was apparent that rbST-treated dairy cows gradually increased feed intake to meet increased metabolic demand (Bauman, 1992). According to Chalupa et al. (1996), DMI were increased ($P<0.05$) by 5.4 and 8.4% for dairy cows injected with 20.6 and 41.2 mg bST/day respectively, in which cows began to increase DMI after 4 weeks of rbST treatment. This increase in DMI coincided with increased milk production. Also, increased FCM production showed that most of the increased DMI accounted for higher milk production. Thus, rbST-treated cows increased DMI in response to increased production in a predictable manner.

When rbST is administered to cows, more nutrients are needed for increased synthesis of milk protein, fat and lactose (Chalupa et al., 1996). Initially, body stores of protein, fatty acids and glycogen may provide additional nutrients, but nutrients for prolonged increases of production are derived from coordinated changes in the metabolism of many tissues and by increased FI (Bauman, 1992). Chapula et al. (1996) also observed that rbST-treated cows produced more ($P<0.01$) FCM per kg of feed consumed. This result showed an improvement ($P<0.01$) in apparent feed efficiency (3.5% FCM/DMI) by 10.4, 12.7 and 12.75% with 10.3, 20.6 and 41.2 mg/day of rbST treatments, respectively.

The mechanism by which rbST might improve FE in dairy cows could be discussed in the light of the following observations. Even though, FI increased with rbST treatment, more dietary nutrients were captured in milk (Bauman, 1992). Because rbST did not affect either digestion of feeds or the efficiency of utilizing absorbed nutrients for milk synthesis (Pillips, 1996), and it has no or little effect on rumen fermentation, flow of nutrients to the small intestine and total tract digestibility (Windsryg et al., 1991), there must be other reasons for the improved FE. When cows produce more milk, they usually consume more feed. The absolute maintenance requirements are not changed, but the relative proportion of intake nutrients needed for maintenance is less and the proportion available for milk synthesis is greater (Chapula et al., 1996). Thus, the relative maintenance requirements are diluted as milk production increases (Chapula et al., 1996). In addition, rbST partitions calories to milk production at the expense of body fat (McGuffy et al., 1991). So, rbST increases the capture of dietary nutrients in milk by decreasing the relative proportion of consumed nutrients needed for maintenance and by partitioning nutrients into milk rather than into body reserves (Chapula et al., 1996). Also, as rbST increased, the weight gain of cows decreased, therefore the main effect of rbST decreased body fat reflecting the partitioning of calories by rbST to milk at the expense of fat deposition (Chalupa et al., 1996). Furthermore, Binelli et al. (1995) using primiparous cows that received rbST (29 mg/day) showed no differences in FI and body weight due to rbST treatment. However, energy balance was lower ($P<0.05$) for rbST-treated than untreated cows. This study indicated that, in the overall process of nutrient partitioning, body weight was not impaired as a consequence of the greater demand for nutrients by the mammary gland in rbST-treated cows. This effect increased mobilization of adipose depots as reflected by decreased body fat and chronic elevation of serum non-estrified fatty acids (Binelli et al., 1995). These changes provide extra energy needed for increasing milk synthesis and lean tissue as observed in lactating primiparous cows (Binelli et al., 1995). In addition, weights of heart, intestine, kidney, liver and lung were greater ($P<0.05$) for rbST-treated than untreated cows (Binelli et al., 1995). Such increases in organ weights in response to rbST treatment probably play a role in increasing the availability of nutrients to the mammary gland and thereby contribute to the galactopoietic effects of rbST in dairy cows. Since the nutrients digestibility or the efficiency of energy utilization for maintenance or milk synthesis were not altered in rbST-treated dairy cows (Schwab and Kirchgessner, 1990), current nutritional standards for lactating cows remain applicable (Chalupa et al., 1996). The most important aspect of nutrition and rbST treatment is that cows must be fed to maximize milk

yield in a given management situation (Pillips, 1996). So, increased dietary protein and energy augmented the milk production responses to rbST at 10.3 mg/day (Austin et al., 1990).

Effect of rbST on physiological functions

Effect of rbST on somatotropic secretion and axis

The effect of rbST administration on bST secretion from anterior pituitary was examined in dairy cows when compared with the effect of rbGRF (growth releasing factor). In this respect, primiparous Holstein dairy cows received rbGRF (12 mg/day) and rbST (29 mg/day) for 63 days (Binelli et al., 1995). Both rbGRF and rbST increased ($P<0.01$) serum bST to concentrations of similar magnitude. Somatotropin content (mg) of pituitary glands from rbGRF- or rbST-treated cows were not different but lower ($P<0.01$) than that of non-treated cows. This support the notion that release of bST from pituitary somatotrphs may have been increased. bST concentrations (mg/g) in pituitary glands and pituitary weight from rbST-treated cows and non-treated were similar. These similarities between rbST-treated and non-treated cows for pituitary weight and bST concentrations suggest that the exogenous bST (rbST) did not inhibit synthesis or release of endogenous bST from anterior pituitary.

Administration of rbST to dairy cows induces several coordinated metabolic changes that subsequently increase milk synthesis. Current believe suggests that insulin-like growth factor (IGF-I) partially mediates rbST-induced increases in milk production (Vanderkool et al., 1995). The bST can act directly on tissues or act indirectly by causing the release of IGF-I (Chase et al., 1998). So, indirect growth-promoting actions of bST are mediated by IGF-I (Puchala et al., 2001). Indeed, IGF-I levels were increased during rbST administration to lactating cows (Molento et al., 2002). The IGF-I peptide is predominantly synthesized and secreted from the liver in response to bST binding with the hepatic bST receptors (Gluckman et al., 1987). The biological effects of IGF-I are further regulated by specific IGF-binding protein that control access of IGF-I to target tissues and by the abundance of the type-I IGF receptor at the target tissues (McGuire et al., 1992). This cascade of events is referred to as the somatotropic axis. In this respect, Vanderkool et al. (1995) showed that both rbST and bGRF similarly increased serum concentrations of ST in cows, and they also increased serum IGF-I, liver IGF-I mRNA and serum IGF-binding protein-3, but serum IGF-binding protein-2, number of free binding sites for IGF-I in mammary tissues were decreased. In liver, rbST did not alter the abundance of mRNA for ST receptors or the number of free binding sites for ST. So, these results suggest that exogenous bST (rbST) is more effective as an IGF-I secretagogue than endogenous bST.

Both bGRF and rbST similarly increased milk yield, suggesting that both hormones stimulate milk synthesis mostly through the same mechanism (Vanderkool et al., 1995). Synthesis of IGF-I in liver is regulated, to a great extent, at the level of mRNA abundance. So, the increased synthesis of IGF-I in liver is largely responsible for the increased serum IGF-I in cows treated with rbST. Despite the fact that rbST stimulated the endocrine IGF-I-BP axis more than bGRF, both hormones stimulated milk synthesis similarly. However, Sharma et al. (1994) reported that serum IGF-I and liver IGF-I mRNA were not correlated with milk yield when cows were in early lactation when compared with those in late lactation. Thus, Vanderkool et al. (1995) concluded that the serum IGF-I is not good indicator for the galactopoietics potency of rbST and rbGRF. Otherwise, the galactopoietic's effects of these hormones are not mediated exclusively through the endocrine IGF-I BP axis. Weber et al. (2007) showed that selection for milk yield increased serum bST, prolonged the postpartum reduction in serum IGF-I. According to Collier et al. (2008), plasma IGF-I and IGF-II were increased in lactating cows treated with rbST (25 mg/d), while milk IGF-I and IGF-II was not affected by rbST treatment. They failed to detect an uncoupling of the somatotropin-IGF-I axis in summer despite an induced negative energy balance during thermal stress.

Effect of rbST on mammary gland

The effect of rbST on mammary function was investigated in dairy cattle when compared with the effect of rbGRF (Binelli et al., 1995). Results on dairy cows that received infusion of rbGRF (12 mg/day) or rbST (29 mg/day) for 63 days, showed that neither rGRF nor rbST affected mammary parenchymal weight or total mammary parenchymal DNA. Such observations are consistent with Capuco et al. (1989) who found no changes in mammary DNA of dairy cows in response to rbST. Binelli et al. (1995) also showed that the total RNA, RNA concentrations, RNA accretion and the RNA to DNA ratio increased in the mammary tissues of cows treated with rbGRF or rbST. Total RNA is an index of cell metabolic activity. Therefore, they suggested that rbGRF and rbST increased the secretory capacity of the mammary gland and their actions on galactopoiesis increased synthesis of milk per mammary cell. Gradual involution of the mammary gland, as reflected by decreasing milk yield, arises from the remodeling transition of mammary epithelial cells from secretory to nonsecretory cells. So, the bGRF and bST increase the metabolic activity of mammary cells or slow their involution without affecting mammary cell numbers or mammary remodeling, thereby allowing more secretory cells to persist over time and contribute to increasing milk synthesis and yield (Binelli et al., 1995). Such interpretation may explain in part the galactopoietic effects of bGRF and bST in dairy cows. Carstens et al. (1997) showed that treatment of rbST at

500 mg/14 days in dairy heifers increased (P<0.01) the proportional weight of fat-free mammary parenchymal tissue by 82%, suggesting that rbST had a great impact on mammary gland development and subsequently milk-producing capacity in dairy heifers.

Effect of rbST on thyroid status

Thyroid hormones are important for maintaining normal lactation. Thyroxine (T_4), the predominant thyroid hormone in blood circulation, has little inherent biological activity, while triiodothyronine (T_3), which is produced by enzymatic 5'-deiodination of T_4 within the thyroid gland, is the most biologically active thyroid hormone (Hadley, 1992). So, the extra-thyroidal activity of 5'-deiodinase (5'D) is an important control to regulate thyroid status of animal tissues in various physiological and pathological situations. Nevertheless, lactating cows are often in a functional hypothyroid state, which is characterized by lower concentrations of circulating iodothyronine and higher concentrations of thyroid-stimulating hormone than those of nonlactating cows. A positive relationship between 5'D activity in the mammary gland and milk yield was reported for rbST-treated cows (Capuco et al., 1989). Short-term administration of bST (40 mg/day) for 5 days to cows in late lactation increased milk yield was accompanied by increased 5'D activity in mammary tissues without changes in 5'D activity in liver and kidney or in the concentrations of serum T_3 and T_4, suggesting that the regulation of extrathyroidal activity of 5'D is also important in lactating cows to permit or mediate the animal galactopoietic responses to rbST treatment (Capuco et al., 1989).

Although, responses of 5'D activity differed among the tissues in the study of Capuco et al. (1989) and Kahl et al. (1995), in both studies, the mammary to liver ratio of 5'D activity was increased for bST-treated cows. Thus, the metabolic priority of the mammary gland could be increased in treated cows either by enhancing general hypo-thyroid status of the cow maintaining euthyroid condition within the mammary tissues (Kahl et al., 1995), or by inducing hyper-thyroid condition in the mammary gland without altering the thyroid status of peripheral tissues or the concentrations of thyroid hormones in the circulation (Capuco et al., 1989). So, the organ-specific responses of 5'D to rbST could be influenced by stage of lactation, season, nutriotional staus, duration, dose and route of rbST administration.

Long-term treatment of lactating dairy cows with rbST influenced thyroid status. In this respect, Kahl et al. (1995) working on primiparous dairy cows received rGRF (12 mg/day) or rbST (29 mg/day) at 118 for 63 days, found that both rGRF and rbST decreased (P<0.001) serum concentrations of T_3 by 10% and the T_3/T_4 ratio by 20%, while serum concentrations of T_4 were not altered due to rbST treatment. These changes in thyroid status were closely related to an increased concentration of serum ST and milk yield (Binelli et al., 1995). The decreased T_3/T_4 ratio for cows treated with somatotropic hormones (rGRF and rbST) suggests that 5'D activity was reduced and was associated with the rapid increase in milk yield as a result of rbST treatment. The reduction in concentrations of circulating T_3 reflected a 30% decrease (P<0.001) in hepatic 5'-deiodinase activity in response to rGRF and rbST. Because the magnitude of changes in 5'D activities and in circulating concentrations of iodothyronines did not differ between bGRF and bST, which had similar concentrations of serum ST, all effects of bGRF on thyroid status can be attributed to effects of the endogenous ST or to ensuing effects of increased lactational intensity. These equivalent effects of bGRF and rbST on milk yield and thyroid hormone status suggest that the mechanisms by which bGRF and bST increase milk yield do not differ substantially.

According to Kahl et al. (1995), thyroid status of lactating cows is regulated by thyroidal secretion of T_4 and T_3 and by the enzymatic 5'-deiodination of T_4 to T_3 in extra-thyroidal tissues. Both rGRF and rbST decreased 5'D activity in liver but did not affect 5'D activity in mammary gland. Such observation indicated a decrease in T_3 generation in liver of rbST-treated cows which could be part of a mechanism whereby energy and nutrients are conserved for use by the stimulated mammary gland. Also, decreased extra-thyroidal conversion of T_4 to T_3 appears to serve as an adaptation to many physiological conditions in which overactive metabolism are undesirable. A reduction in circulating T_3 may induce alterations in nutritional flux such as increasing lipolysis in fat depots (Blennemann et al., 1992), which may provide additional nutrients for lactation. The constant generation of T_3 in mammary tissue, in response to rbST treatment, would provide T_3 to synergize with other galactopoietic hormones at the level of mammary gland. In the case of T_4, Kahl et al. (1995) showed that rbST-treated cows tended to have higher concentrations of T_4 than control. They attributed these increases in T_4 to the decreased extra-thyroidal deiodination of T_4 to T_3.

On the other hand, Kahl et al. (1995) observed that bGRF and rbST treatments did not alter T_3 concentrations in milk. Because generation of T_3 by the mammary gland is a significant source of T_3 in bovine milk, the mammary gland of rbST-treated cows was able to maintain a euthyroid condition despite diminished availability of circulating T_3. This indicates that secretion of T_3 into milk could not account for the lower serum T_3 in rbST-treated cows. So, the galactopoietic effects of rbST and rbGRF were due to increased metabolic activity of secretory cells rather than to an increase in the number of mammary cells (Binelli et al., 1995). Accordingly, stimulation of milk production by rbST (Binelli et al., 1995)

is supported by changes in local thyroid status (Kahl et al., 1995). Taken together, it is suggested that bGRF and rbST increase the hypothyroid status of the lactating cows and maintain euthroid condition in the mammary gland, thus enhancing the metabloic priority of the mammary gland. A study carried on cows that received 29 mg/day rbST, indicated that increased substrate required for enhanced milk fatty acids yield may have been provided through redirection of nutrients to the mammary gland away from adipose tissue and through overall increased metabolism in the mammary gland (Beswick and Kennelly, 2000).

Effect of rbST on reproduction

Many studies have evaluated the effects of rbST on dairy animal reproduction. These effects of rbST on reproduction were related to rbST dose, time of initiation of treatment, time of initiation of breeding and control of other factors such as nutritional status and milk production of cows (Esteban et al., 1994). Phillips (1996) stated that reproductive variables have been focused on production studies including days to open (parturition to concenption interval), days to first service (interval from parturition to first insemination) and services per conception (number of inseminations required to achieve conception). In addition, rbST has effects on the function of the ovarian granulosa cells *in vitro* (Langhout et al., 1991) and on ovarian function *in vivo* (De la Sota et al., 1993).

Rajamahendran et al. (1989) showed that using rbST (0, 10.3 or 20.6 mg) in Holstein cows from 35 days of lactation until 70 days prior to calving, based on milk progesterone, had no effects on reproductive variables such as days to first ovulation, number of cows with short first cycles, mean cycle length in days, mean peak luteal activity respectively due to rbST treatments. Also, mean days to first observed oestrus, average days open, number of pregnant at 200 days post-calving and incidences of reproductive disorders were not affected by rbST treatments. However, cows receiving 10.3 mg of rbST required fewer services per conception (1.30) than 0 (1.75) or 20.6 mg (1.81) cows. Meanwhile, Nytes et al. (1990) working on dairy cows given 0, 10.3, 20.6 or 30.9 mg bST/day from 14th week after parturition for 30 weeks, observed no noticeable effects on reproductive performance due to rbST. Similar response was found in cows given rbST (0, 100, 200, 300, 400 or 780 mg) commenced at 110 days postpartum and continued until cows dried off (Burchard et al., 1990).

Esteban et al. (1994) focused on the reproductive performance of dairy cows treated with rbST (17.2, 51.6 and 86 mg/day) which started at 70 days postpartum and ended at dry-off for 2 consecutive lactations. During the first lactation, multiparous cows treated with rbST decreased ($P<0.05$) pregnancy rates, increased behavioral anestrus and anestrus confirmed by palpation.

Such effect was not observed in primiparous cows or in multiparous cows in the second lactation. Meanwhile, rbST-treated primiparous cows had ($P<0.05$) shorter mean days to first standing estrus in the first lactation. In the second lactation, rbST-treated cows had an increased delayed uterine involution, cystic ovarian condition, behavioral anestrus and anestrus confirmed by palpation. However, no differences was detected in progeny from the treated cows of first lactation as evaluated for rates of growth, morbidity, mortality and for reproductive performance. At the same time, Esteban et al. (1994) reported that because rates of dystocia and retained fetal membranes increased in control and rbST-treated cows, exposure to rbST was probably not directly related to those changes. The observation that rbST did not increase the risk for a cow developing cystic structures during the early postpartum period of the following lactation agreed with similar finding reported by Cole et al. (1992). Also, Lucy et al. (1991) noticed that rbST may have a direct effect on ovarian tissues. This effect was described as an increase in the number of primary follicles recruited with a subsequent inability of the mature Graafian follicle to show complete dominance as reported by Esteban et al. (1994), who concluded that before definite conclusions are made regarding reproductive performance, repercussions in milk production, energy balance, body condition score and blood metabolites that arise with the use of rbST should be considered. At this point, it has been concluded that rbST treatment at a level of 250 mg at 14-day interval for five successive times pre-puberty is strongly in relation to concentration of GH and IGF-I and in less extent to glucose and urea-N concentration to induce precocious puberty in Friesian heifers (Gabr, 2013).

Many studies have investigated the effects of rbST on ovarian function in dairy cattle. Spicer and Stewart (1996), in an *in vitro* study to determined whether rbST affect basal estradiol, progesterone and androstenedione produced by granulosa cells of small and large follicles, found that pharmacologic doses of rbST (300 ng/ml) inhibited ($P<0.05$) estradiol, but physiologic dose of rbST (50 ng/ml), bST had no effect on estradiol production by granulosa cells of small or large follicles. Also, rbST blocked the increase in estradiol production that had been induced by FSH, but it had no effect on progesterone production by granulosa cells or thecal cells of large follicles. Also, Gong et al. (1994) reported similar results, but they found that rbST stimulated basal progesterone and FSH-induced progesterone production by bovine ovarian granulosa cells from large follicles (>10 mm). The rbST, at physiologic doses, could either stimulate or inhibit androstenedione production by thecal cells of large follicles depending on whether thecal cells responded to luteinizing hormone. This finding indicated that rbST might have indirectly affected estradiol production by influencing aromatizable estrogen precursors. In addition to its inhibitory effects on steriod production,

rbST also inhibited proliferation of granulosa and thecal cells from large follicles. This effect could be related to the observation that rbST inhibited incorporation of [^{3}H] thymidine, a measure of DNA synthesis, by bovine granulosa cells from large follicles (>10 mm) as repoted by Gong et al. (1993).

The foregoing discussion indicated that the magnitude of reproductive responses of dairy cows to rbST is variable (Phillips, 1996). High doses of rbST treatment (20.6 mg/day) decreased conception rates, increased days open by 28-30%, days to first estrus and twinning rates and there was a trend for increased services per conception (Burton et al., 1990b). However, several studies dealt with the rbST treatments which found no differences in days open, services per conception and days to first estrus at 500 mg/14 day (Weller et al., 1990; Pell et al., 1992) or at 56-700 mg/14 day (Downer et al., 1993). In addition, increases in days open were observed in cows in which rbST treatment was initiated early in lactation, but not when treatment started at mid or late lactation (McGuffey et al., 1991). In a more recent study, Abdel-Rahman et al. (2010), working on Holstein-Friesian cows, found that the days-open showed non-significant differences between subgroups for primiparous cows while there was apparent increase in multiparous cows receiving 250 and 500 mg bST/14 days. They also detected an improvement of conception rate (%) in all treated cows as compared to the control. Furthermore, Chalupa et al. (1996) working on dairy cows injected with rbST (10.3, 20.6 or 41.2 mg bST/d) at d 28 to 35 of lactation, observed that 41.2 mg/day of rbST reduced ($P<0.05$) pregnancy rate, but did not affect AI services, conception rate and days open. However, rbST at 10.3 or 20.6 mg/day did not adversely affect reproduction. Flores et al. (2007) reported that rbST at 500 mg/14 day in Brahman cows increased ($P<0.05$) the first-service conception rate during the first 30 days of breeding and pregnancy rates during the first 3 days of breeding. In addition, the success of embryo transfer in cattle can be compromised by low pregnancy rates. The use of rbST in lactating dairy cows increased pregnancy rates following the transfer of frozen-thawed embryos (Moreira et al., 2002), and this effect may be mediated directly by rbST or indirectly by IGF-I (de la Sota et al., 1993).

The observed decreases in reproductive performance of dairy cattle treated with rbST may be attributed more to the increases in milk yield and short-term negative energy balance than to direct effects of rbST (Weller et al., 1990). According to Phillips (1996), days open was more related to level of milk production than rbST. In addition, treatment with rbST increases milk energy output before there is a concomitant increase in feed intake and therefore, following initiation of rbST treatment, treated cows tend to be in more negative energy balance which is known to reduce reproductive performance. In the early post-partum period of dairy cows, the duration and intensity of negative energy balance, the level of body condition score loss and the milk yield are strongly associated with the timing of the first ovulation. In this respect, pre-partum administration of rbST (500 mg) in late pregnant Holstein heifers did not affect the milk production, plasma β-hydroxybutyrate and non-esterified fatty acids concentrations, the time of the first post-partum ovulation and the proportion of cows ovulating the first post-partum follicular wave showes that pre-partum rbST treatment in dairy heifers with high body condition score seems not to have any effect on markers of energy balance, milk production or development of the first follicular wave in the early post-partum period (Acosta et al., 2013).

Effect of environmental conditions on animal response to rbST

Several studies have documented the negative effects of hot or cold conditions on milk production. Responses of milk yield to rbST treatments during heat stress appear to be smaller than under more moderate conditions (Staples et al., 1988).

Working on Holstein lactating cows at 46-106 days postpartum, Zoa-Mboe et al. (1989) evaluated the effects of rbST injection (20.6 mg/d) and environment (shade and no shade systems) on milk production and physiological functions of dairy cows. The ambient temperature ranged between 21.2-32.3°C and humidity between 50-90%. The rectal temperature (RT) and respiration rate (RR) of cows were higher ($P<0.05$) in no shade than in shade. The rbST-treated cows had slightly higher ($P<0.05$) RT and RR. This increase in RT and RR might have resulted from increased metabolic heat production, and this would increase slightly the need of rbST-treated cows to dissipate their body heat. However, comparable increases in RT and RR of rbST-treated cows were not observed under more moderate environmental conditions during full lactation (Soderholm et al., 1988). The rbST supplementation with 500 mg/14 day in crossbred Holstein cattle under hot and humid condition affected body acid-base homeostasis (Thammacharoen et al., 2011).

Zoa-Mboe et al. (1989) also observed that yields of milk and 3.5% FCM and DMI of cows in no shade were 9.5 and 16% less ($P<0.05$) than for cows in shade. Much of the effect of heat stress on milk production was associated with reduced DMI. The rbST injection increased ($P<0.05$) 3.5% FCM and milk component yields, while milk yield was increased insignificantly by 4.5% (1.1 kg/d). DMI and gross efficiency (FCM/DMI) were unaffected by rbST treatment. The two environmental systems did not affect plasma metabolites and hormones (glucose, NEFA, insulin, prolactin, bST, T_3 and T_4). Also, rbST treatment had no significant effect on plasma constituents, except rbST-injected cows which had greater ($P<0.001$) concentrations of plasma bST. It

seems apparent that cows in shade or no shade systems were impacted by heat stress, and this might explain why responses of cows to rbST were not different under the two environmental systems (Zoa-Mboe et al., 1989).

On the other hand, Becker et al. (1990) observed that short-term injection of rbST in cows kept under cold stress of ambient temperature ranging from -5 to 5°C increased their milk yield. Also, milk production was increased for rbST-treated Holstein and Jersey dairy cows exposed to hot conditions in which ambient temperature ranged 22-35°C and humidity 30 to 100% (West et al., 1990; Johnson et al., 1991). However, Zoa-Mboe et al. (1989) reported that increased production responses to rbST in heat-stressed dairy cows were less than in cows injected with rbST under more moderate conditions. Thus, just as milk yield is reduced in non-treated cows maintained under heat stress conditions, milk yield response to rbST is also reduced (Philips, 1996). Reasons may be related to need of dairy cows to maintain their body core temperature by decreasing heat production and increasing insensible heat loss. The rbST treatment (31 mg) increased milk production similarly during the thermoneutrality and heat-stress periods, ~8.3% over the control and this increase occurred together with the elevation in core body temperature (Settivari et al., 2007). It has been suggested that exogenous rbST is efficacious in increasing milk yield without adverse effects on lactating crossbred Holstein cows in a tropical environment (Chaiyabutr et al., 2011). Under tropical conditions, it has been demonstrated that the rbST exerts its galactopoietic action, in part, through increases in total body water, empty body water and extracellular water in association with an increase in mammary blood flow, which partitions the distribution of nutrients to the mammary gland for milk synthesis in crossbred cattle (Chaiyabutr et al., 2007). Therefore, strategies to overcome heat stress of moderate and high producing dairy cows should permit greater benefits to be realized from use of rbST in tropical and subtropical environmental conditions (Zoa-Mboe et al., 1989).

Effects of rbST on animal health and immune system

Effects of rbST treatment on the health of dairy cows have been reviewed (Phillips, 1996). As observed by Soderholm et al. (1988), adminstration of rbST to cows did not influence their body temperature or respiration rate, but heart rate increased by 5-15%. Although, Burton et al. (1990b) observed no significant increase in leg or hoof problems in rbST-treated cows, Cole et al. (1992) and Zhao et al. (1992) reported increased incidence of of lameness in rbST-treated cows. Meanwhile, some metabolic diseases such as milk fever or ketosis did not affect rbST treatment (Bauman, 1992). Also, Nytes et al. (1990) observed no adverse affect on health or incidence of disease due to rbST. Chalupa et al. (1996) showed that the infertility was about 3 times greater for 41.2 mg, rbST treated-cows than for untreated, but there was no indication (P>0.10) of increases in pregnancy failure incidence of ketosis, abomasal displacement, feet and leg problems or clinical mastitis in 10.3 or 20.6 mg bST-treated cows. However, 41.2 mg bST-treated cows tended to have more feet and leg problems and required antibiotic for mastitis and have higher somatic cell counts (SCCs) in milk.

According to Phillips (1996), the effects of rbST on udder health and the incidence of mastitis are important. So, many studies examined the number of clinical cases of mastitis that coincided with the treatment of rbST. Also, SCCs in milk have been evaluated as an indirect index of subclinical mastitis. However, the effect of rbST on SCC and mastitis is variable. Some studies indicated that SCC increased in rbST-treated cows (Oldenbroek and Garssen, 1990), but others found no differences in SCC in treated and non-treated cows (Zinn et al*.,* 1993). In case of mastitis, no significant effect on incidence of mastitis and health problems was seen for cows given rbST at doses from 100 to 780 mg. However, increased incidence of mastitis appears to be more related to random effects than specifically to rbST (Pell et al., 1992). Thus, White et al. (1994) found no association between rbST treatment and the incidence or duration of clinical mastitis. They concluded that under normal conditions, there is a positive relationship between the incidence of mastitis and peak and total milk yield and that treatment with rbST did not alter this relationship. So, cows that produce more milk normally have a greater tendency to develop mastitis and this relationship exists regardless of the use of rbST (White et al*.,* 1994). Accordingly, the incidence of mastitis in rbST-treated cows is due more to the increased milk yield than to any direct effects of rbST (Phillips, 1996).

Immune status of lactating cows given rbST (10.3 and 20.6 mg/day) for 38 weeks was studied by Burton et al*.* (1990a) who found that rbST treatment at 10.3 mg/day increased IgG and IgG2 by 12.4 and 18.4%, respectively than the control. At dosages that are efficacious for lactation, rbST is not detrimental to humeral immune or cell-mediated immune responses as determined by serum concentrations of IgG, IgG1, IgG2 and IgGA. Additionally, in primiparous and multiparous cows given rbST doses at 250 and 500 mg/14 days, the leucogram showed a non significant leucocytosis accompanied with neutrophilia and lymphocytosis (Abdel-Rahman et al., 2010).

EFFECT OF rbST ON LACTATING SHEEP AND GOATS

The galactopoietic effects of bST are well established in sheep (Stelwagen et al., 1993) and dairy goats (Knight, 1992). However, a few studies have investigated the effect of rbST on milk production in lactating dairy ewes and goats. The milk yield responses to rbST treatment in

goats and sheep are more variable than that in cows (Davis et al., 1999). The rbST has been shown to be active in goats (Puchala et al., 2001).

In the case of sheep, Fernandez et al. (1995) used 74 lactating dairy ewes injected with rbST at 0, 80, 160 or 240 mg every 14 days from 3 to 8 weeks of lactation (part 1) and 0, 80 or 160 mg every 14 days from 11 to 23 weeks of lactation (part 2). They showed that rbST treatment increased milk yield ($P<0.01$) at all treatments over the control. The largest increase in milk yield was at 160 mg of bST by which milk yields increased by 34.1 and 53.2% and 6% FCM by 36.9 and 51.8% for parts 1 and 2, respectively. The rbST increased milk fat during part 1, but decreased milk fat during part 2. Protein content of milk was decreased while yield of milk constituents were increased. The rbST did not affect milk lactose during part 1, but increased milk lactose in part 2. Milk yield constituents were increased due to rbST treatments. Fernandez et al. (1995) also showed that neither mastitis incidence nor milk SCC was affected by bST treatment. Therefore, they concluded that bST is efficacious in increasing both actual milk yield and 6% FCM over the dose range of 80 to 240 mg/14 days without adverse effects on lactating ewes. Bassett et al. (1998), working on lactating sheep, confirmed the galactopoietic effects of rbST (0.1 mg/kg/day) and suggested that the mechanism of this action is not via increased hepatic growth hormone receptor number or gene expression. They added that the increase in hepatic but not mammary IGF-I mRNA with rbST treatment suggests an endocrine action of IGF-I on milk synthesis. In a recent study, Requena et al. (2010) determined the effect of administration of rbST (40, 80 and 120 mg/14 day) in lactating ewes. They found that the treatment 120 mg of bST yielded 39% more actual milk and 44 more 6% FCM; and increased actual milk fat percentage, but did not affect the other milk components or somatic cell count, concluding that rbST increased potential milk yield throughout lactation and actual milk yield only after weaning in dairy ewes, however it was not useful for reducing the milk yield loss that occurred at weaning. With rbST dose titration and treatment interval in lactating dairy ewes, Fernandez et al. (2001) concluded that the period between successive injections should be shorter, the dose employed lower; however, a 14-day period seemed to correspond correctly to the dosages and hormone formulation tested. Even though exogenous administration of rbST effectively increases milk yield, more permanent and profitable results can be achieved by enhancing technical practices, focusing on better genetic goals for dairy sheep, and taking care of udder health (Pulina et al., 2007). On the other hand, injection with 100 mg rbST/biweekly increased the average daily gain and improved the physiological status of growing post weaning growing Rahmani lambs (Nour El-Din et al., 2009). In addition, administration of 80 mg rbST/lamb, at 14-days interval starting at 2 month of age for both male and female lambs produced from crossbreed ewes (½ Finnish Landrace x ½ Rahmani) previously treated with 160 mg rbST/ewe, improved growth performance and some puberty characteristics in male lambs and slightly improved age at puberty in ewe lambs (El-Gohary et al., 2011).

In the case of goats, Davis et al. (1999) using 14 multiparous Angora does injected with rbST (100 µg/kg BW/d), showed that body weight and dry matter intake of does were not affected by rbST treatment. Kids of rbST-treated does had higher ($P<0.05$) average daily gain (ADG) by 32% than the kids of control, suggesting that kid ADG is probably a good indicator of actual milk production. However, milk yield was insignificantly increased by 15% for rbST-treated does than control. These increases in milk yield and ADG of the kids of rbST-treated does without change in DMI or BW of does are consistent with similar responses in milk yield by lactating cows treated with rbST without corresponding increase in DMI (Downer et al., 1993; Bareille et al., 1997). No carryover effect of bST on milk production was observed in goats (Knight, 1992; Davis et al., 1999). The lack of change in milk composition (fat, protein, lactose and total solids) in rbST-reated goats observed by Davis et al. (1999) is consistent with previous report on goats (Nielsen, 1988) and cattle (Dahl et al., 1993). Furthermore, in rbST-treated goats (Davis et al., 1999), concentrations of plasma NEFA, total protein, glucose, insulin, urea and amino acids were not affected by rbST treatment. Such observation in goats disagree with study of Sechen et al. (1989a), who reported that treatment of cows with bST increased milk yield and plasma concentrations of NEFA, but that there were no changes in plasma glucose, insulin and glucagon. In the study of Sechen et al. (1989a), the increase in milk energy secretion made the cows to be in negative energy balance, which resulted in a chronic elevation of circulating NEFA. Changes in plasma concentrations and increased oxidation of NEFA have been reported in dairy cows that were in negative energy balance during bST treatment (Sechen et al., 1989b). The unchanged concentrations of plasma amino acids agree with similar trend observed by Davis et al. (1995).

Plasma concentrations of T_4 and cortisol were decreased, while plasma ST and IGF-I were elevated ($P<0.05$) chronically in bST-treated goats (Davis et al., 1999). Similar trend of plasma ST and IGF-I were reported by Davis et al. (1999) in goats injected daily with bST (100 µg/kg BW/day). In this respect, Prosser et al. (1990) reported increase in milk yield of goats which coincided with increased plasma concentrations of IGF-I. There is a complex interaction between rbST administration and thyroid hormone status in Angora goats as reported by Puchala et al. (2001) who showed that in the case of hypothyroid, rbST increased plasma concentrations of T_3 and T_4, however, in the cases of euthyroid or hyperthyroid, rbST had no effect on thyroid hormones.

These findings indicate that rbST maintained the normal thyroid status. A study on Damascus lactating goats in their third to fourth lactation season and at 30-40 days postpartum were subcutaneously and biweekly received an injection for 8 weeks with low (50 mg/doe) or high (100 mg/doe) doses of rbST, Sallam et al. (2005) found that the DMI or BW of does were not affected significantly by rbST treatment, while ADG of kids suckling rbST-treated does was higher by 11.0 and 10.5% for low and high doses of rbST, respectively than for kids of control does. They also found that administration with rbST resulted in a significant ($P<0.05$) total milk yield by 24.3 and 22.5% for 50 or 100 mg rbST, respectively when compared with the control. There were insignificant increase in the levels of total solids, milk protein and fat, while lactose was significantly ($P<0.05$) increased due to rbST exposure. Sallam et al. (2005) also showed that ash, hematological parameters, plasma protein and the activities of plasma AST, ALT, glutathione S-transferase, alkaline phosphatase, acid phosphatase and the levels of thiobarbutric acid-reactive substances, creatinine and cholesterol were not affected by the rbST treatments, while administration of rbST significantly ($P<0.05$) increased plasma glucose levels and decreased those of urea and total bilirubin. Injection of rbST at 20, 40 and 60 mg/14 days to West African Dwarf goats increased their milk yield by 50.4, 68.0 and 71.2%, respectively, suggesting that rbST administration to goats after peak of lactation can enhance milk yield, galactopoiesis and persistency of lactation, indicating higher milk yield in extended lactation (James et al., 2010). Recently, the treatment of lactating goats with rbST rapidly increased milk yield after the onset of treatment according to Qudus et al. (2013). They reported that, injection with 50 and 100 mg/week of rbST for 8 weeks in lactating goats increased milk production by 28 and 29%, respectively, accompanied by no significant effect on milk composition and without any adverse effect on health of goats. Similar results were previously observed by Chadio (2009) in lactating goats.

CONCLUSION

The rbST has a galactopoietic effect which improves milk production in dairy animals without adverse effect on milk quality or animal health and reproduction. The mechanistic aspects that involved the positive effects of rbST could be included which increased the metabolic activity of mammary cells or slow their involution, without affecting mammary cell numbers or mammary remodeling, thereby allowing more secretory cells to persist over time and contribute to increasing milk synthesis and yield. Furthermore, rbST increase the hypothyroid status of the lactating cows and maintain euthroid condition in the mammary gland, thus enhancing the metabolic priority of the mammary gland. With rbST treatment, the increased substrate required for enhanced milk fatty acids yield may have been provided through redirection of nutrients to the mammary gland away from adipose tissue and through overall increased metabolism in the mammary gland. As well, rbST increases the capture of dietary nutrients in milk by decreasing the relative proportion of consumed nutrients needed for maintenance and by partitioning nutrients into milk rather than into body reserves, therefore, the main effect of rbST was decreased body fat reflecting the partitioning of calories by rbST to milk at the expense of fat deposition.

REFERENCES

Abdel-Rahman HA, Khalil AS, EL-Hamamsy HT, Ezzo OH (2010). The Effect of recombinant bovine somatotropin administration on milk production, some hemato- biochemical parameters and reproductive performance of lactating cows. Global Veterinaria 4:366-373.

Acosta DAV, Pfeifer LFM, Schmitt E, Schneider A, Silveira PAS, Jacometo CB, Brauner CC, Rabassa VR, Corre MN, Del Pino FAB (2013). Effect of pre-partum somatotropin injection in late pregnant Holstein heifers with high body condition score on metabolic parameters, resumption of ovulation and milk production. Can. J. Anim. Sci. 93:287-292.

Austin CL, Schingoethe DJ, Casper DP, Cleale RM (1990). Interaction of bovine somatotropin and nutrition on milk production and composition from dairy cows. J. Dairy Sci. 73:Suppl. 1, 159.

Barbano DM, Lynch JM, Bauman DE, Hartnell JF, Hintz RL, Memeth MA (1996). Effect of prolonged-release formulation of n-methionyl bovine somatotrophin (sometribove) on milk composition. J. Dairy Sci. 75:1775.

Bareille N, Faverdin P, Hay M. (1997). Modification of feed intake response to $ß_2$-agonist by bovine somatotropin in lactating or dry cows. J. dairy Sci. 80:52.

Bassett NS, Currie MJ, Breier BH, Klempt M, Min SH, McCutcheon SN, MacKenzie DD, Gluckman PD. (1998). The effects of ovine placental lactogen and bovine growth hormone on hepatic and mammary gene expression in lactating sheep. Growth Horm. IGF Res. 8:439-446.

Bauman DE (1992). Bovine somatotropin: Review of an emerging animal technology. J. Dairy Sci. 75:3432.

Bauman DE (1999). Bovine somatotropin and lactation: from basic science to commercial application. Domestic Anim. Endocrinol. 17:101-116.

Becker BA, Johnson HD, Li R, Collier RJ (1990). Effect of farm and stimulated laboratory cold environmental conditions on the performance and physiological responses of lactating dairy cows supplemented with bovine somatotropin (BST). Internat. Biometeoriol. 34:151.

Beswick NS, Kennelly JJ (2000). Influence of bovine growth hormone and growth hormone-releasing factor on messenger RNA abundance of lipoprotein lipase and stearoyl-CoA desaturase in the bovine mammary gland and adipose tissue. J. Anim. Sci. 78:412.

Binelli M, Vanderkool WK, Chapin LT, Vandhaar MJ, Turner JD, Moseley WM, Tucker HA (1995). Comparison of growth hormone-releasing factor and somatotropin: Body growth and lactation of primiparous cows. J. Dairy Sci. 78:2129.

Blennemann BY, Moon YK, Freake HC (1992). Tissue-specific regulation of fatty acids synthesis by thyroid hormone. Endocrinol. 190:637.

Burchard JF, Gallo M, Mckiddie D, Block E. (1990). Effect of bovine somatotropin on milk yield in dairy cows. Brief Communication of the XXIII International Dairy Congress, Montreal, October 8-12. Vol. I.

Burton JL, McBride BW, Kennedy BW, Burton JH, Elsasser TH, Woodward B (1990a). rbST and the bovine immune system. Brief Communication of the XXIII International Dairy Congress, Montreal, October 8-12. Vol. I, 80.

Burton JL, McBride BW, Burton JH, Eggert RG (1990b). Health and reproductive performance of dairy cows treated for up to two consecutive lactations with bovine somatotropin. J. Dairy Sci. 73:

3258.
Capper JL, Castañeda-Gutiérrez E, Cady RA, Bauman DA (2008). The environmental impact of recombinant bovine somatotropin (rbST) use in dairy production. PNAS, 105:9668-9673.
Capuco AV, Keys JE, Smith J.J. (1989). Somatotropin increases thyroxine-5'-monodeiodinase activity in lactating mammary tissue of the cow. J. Endocrinolgy 121:205.
Carstens GE, Glaser DE, Byers FM, Greene LW, Lunt DK (1997). Effects of bovine somatotropin treatment and intermitten growth pattern on mammary gland development in heifers. J. Anim. Sci. 75:2378-2388.
Chadio S (2009). Effects of recombinant bovine somatotropin administration to lactating goats. Small Rumin. Res., 35:263-269.
Chaiyabutr N, Boonsanit D, Chanpongsang S (2011). Effects of cooling and exogenous bovine somatotropin on hematological and biochemical parameters at different stages of lactation of crossbred Holstein Friesian cow in the tropics. Asian-Aust. J. Anim. Sci. 24:230-238.
Chaiyabutr N, S Thammacharoen, S. Komolvanich, S. Chanpongsang. (2007). Effects of long-term exogenous bovine somatotropin on water metabolism and milk yield in crossbred Holstein cattle. J. Agric. Sci. (Cambridge) 145:173-184.
Chalupa W, Vecchiarelli B, Galligan DT, Verguson JD, Baird LS, Hemken RW, Harmon RJ, Soderholm CG, Otterby DE, Annexstad RJ, Linn JG, Hansen WP, Ehle FR, Palmquist DL, Eggert RG (1996). Responses of dairy cows supplemented with somatotropin during weeks 5 through 43 of lactation. J. Dairy Sci. 79:800.
Chase CC Jr, Kirby CJ, Hammond AC, Olson TA, MC Lucy (1998). Pattern of ovarian growth with a growth hormone receptor deficiency. J. Anim. Sci. 76:212.
Cole WJ, Eppard PJ, Boysen BG, Madsen KS, Sorbet RH, Miller MA, Hintz RL, White TC, Ribelin WE, Hammond BG, Collier RJ, Lanza GM (1992). Responses of dairy cows to high doses of a sustained-release bovine somatotropin administered during two lactations. J. Dairy Sci. 75:111.
Collier RJ, Miller MA, McLaughlin CL, Johnson HD, Baile CA (2008). Effects of recombinant bovine somatotropin (rbST) and season on plasma and milk insulin-like growth factors I (IGF-I) and II (IGF-II) in lactating dairy cows. Domestic Anim. Endocrionol. 35:16-23.
Dahl GE, Chapin LT, Allen MS, Moseley WM, Tuker HA (1993). Galactopoietic effects of recombinant somatotropin and growth hormone-releasing factor in dairy cows. J. Dairy Sci. 76:1550-1557.
Davis JJ, Sahlu T, Puchala R, Herselman MJ, Fernandez JM, McCann JP, Coleman S.W. (1999). The effect of bovine somatotropin treatment on production of lactating Angora does with kids. J. Anim. Sci. 77:17.
De La Sota RL, Lucy MC, Staples CR, Thacher WW (1993). Effects of recombinant bovine somatotropin (sometribove) on ovarian function in lactating and nonlactating dairy cows. J. Dairy Sci. 76:1002-1013.
Downer JV, Patterson DL, Rock DW, Chalupa WV, Cleale RM, Firkins JL, Lynch GL, Clark JH, Brodie BO, Jenny BF, De Gregorie R (1993). Dose titration of sustained-release recombinant bovine somatotropin in lactating dairy cows. J. Dairy Sci. 76:1125.
El-Gohary E S, Abdel-Khalek EA, Ashmawy TAM, Teleb DF, Sallam A A (2011). Effect of recompinant bovine somatotropin (rbST) on growth performance and puberty incidence of male and female lambs born from rbST treated ewes. Egyptian J. Sheep Goat Sci. 6:47 -57.
Esteban E, Kass PH, Weaver LD, Rowe JD, Holmberg CA, Franti CE, Fred Troutt H. (1994). Reproductive performance in high producing cows treated with recombinant bovine somatotropin. J. Dairy Sci. 77:3371.
Fernandez N, Molina M P, Balasch S, Torres A, and Adriaens F. (2001). Bovine somatotropin dose titration in lactating dairy ewes. 3. Treatment interval. J. Dairy Sci. 84:2170-2176.
Fernandez N, Rodriguez M, Peris C, Barceld M, Molina MP, Torres A, Adriaens F (1995). Bovine somatotropin titration in lactating dairy ewes. 1. Milk yield and milk composition. J. Dairy Sci. 78:1073.
Flores R, Looper ML, Rorie RW, Lamb MA, Reiter ST, Hallford DM, Krieder DL, Rosenkrans CF (2007). Influence of body condition and bovine somatotropin on estrous behavior, reproductive performance, and concentrations of serum somatotropin and plasma fatty acids in postpartum Brahman-influenced cows. J. Anim. Sci. 85:1318.
French N, Boer G, Kennelly JJ, Boer G. (1990). Effects of feeding frequency and exogenous somatotropin on lipolysis, hormone profils and milk production in dairy cows. J. Dairy Sci. 73:1552.
Gabr Sh A (2013). Effect of rbST administration on pubertal age and some blood parameters in Friesian heifers. Egyptian J. Anim. Prod. 50:122-131.
Gallo GF, Block E (1990). Effects of recombinant bovine somatotropin in nutritional status and liver function of lactating dairy cows. J. Dairy Sci. 73:3276.
Gibson JP, M. Muelen Van der, McBride BW, Burton JH (1992). The effects of genetic and phenotypic production potential on response to recombinant bovine somatotropin. J. Dairy Sci. 75:878.
Gluckman PD, Breier BH, Davis SR (1987). Physiology of the somatotropic axis with particular reference to the ruminant. J. Dairy Sci. 70:442.
Gong J, Baxter G, Barmley T, Webb R (1997). Enhancement of ovarian follicle development in heifers by treatment with rbST: A dose response study. J. Reprod. Fertil. 110:91.
Gong JG, McBride D, Bramley TA, Webb R (1993). Effects of recombinant bovine somatotropin, insulin like growth factor-I and insulin on the proliferation of bovine granulose cells *invitro*. J. Endocrinol. 139:67
Gong JG, McBride D, Bramley TA, Webb R (1994). Effects of recombinant bovine somatotropin, insulin like growth factor-I and insulin on bovine granulosa cell steroid genesis *in vitro*. J. Endocrinol. 143:157.
Gulay MS, Hayen MJ, Teixeira LC, Wilcox CJ, Head HH (2003). Responses of Holstein cows to a low dose of somatotropin (bST) prepartum and postpartum. J. Dairy Sci. 86:3195-3205.
Hadley ME (1992). Endocrinology. 3rd Ed. Prentic Hall Interbational Inc. Englewood Cliffs, New Jersey. USA.
Huber JT, Sullivan JL, Willman S, Arana M, Denigan M, De Corte C, Hoffman RG, Hartnell GF (1991). Response of Holstein cows to biweekly sometribove (SB) injections for 4 consecutive lactations. J. Dairy Sci. 74 (Suppl. 1):211 (Abstr.).
James IJ, Osinowo OA, Smith O F, Bemji MN, Rekwot PI (2010). Effects of bovine somatotropin on the pattern of milk yield within and between injection intervals in west African dwarf goats. J. Agric. Sci. Environ. 10:86-97.
Johnson HD, Li R, Manalu W, Spencer-Johnson KJ, Becker BA, Coiler RJ, Baile CA (1991). Effects of somatotrophin on milk yield and physiological responses during summer farm and hot laboratory conditions. J. Dairy Sci. 74:1250.
Kahl S, Capuco AV, Binelli M. Vanderkool WK, Tucker HA, Moseley WM (1995). Comparison of growth hormone-releasing factor and somatotropin: Thyroid status of lactating primiparous cows. J. Dairy Sci. 78:2149.
Kannan CR (1987). The pituitary gland. Plenum Publishing Corp. New York.
Kazmar GW, Canfield RC, Bean B (1990). Plasma growth hormone and prolactin concentrations in young dairy sires before and after 1 24 h fast. J. Dairy Sci. 73:3112.
Knight CH (1992). Milk yield responses to sequential treatments with recombinant bovine somatotropin and frequent milking in lactating goats. J. Dairy Res. 59:115.
Langhout DJ, Spicer LJ, Geisert RD (1991). Development of a culture system for bovine granulosa cells effects of growth hormone, estradiol and gonadotropins on cell proliferation, steroidgenesis and protein synthesis. J. Anim. Sci. 69:3321.
Laurent F, Vignon B, Coomants D, Wilkinson J Bonnel A (1992). Influence of bovine somatotropin on the composition and manufacturing properties of milk. J. Dairy Sci. 75:2226.
Lucy MC, Staples CR, Michel FN, Thacher WW (1991). Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum cows. J. Dairy Sci. 74:473.
Ludwig SKJ, Smits NGE, Van der Veer G, Bremer MGEG, Nielen MWF (2012). Multiple Protein biomarker assessment for recombinant bovine somatotropin (rbST) abuse in cattle. PLOS ONE 7 (12):e52917.
Madakaze CR, Phipps RH, Hard DL (1990). Preliminary results on the influence of bovine somatotropin (sometribove) on milk production in Zimbabwe. J. Dairy Sci. 73 (Suppl. 1):161 (Abstr.).

Mattos WA, AV Pires, de Varis VP, Doque JD, Madsen KS (1989). The effect of sometribove (recombinant mrthyionyl bovine somatotropin) on milk yields and milk composition in lactating dairy cows in Brazil. J.Daiiry Sci. 72 (Suppl. 1):452 (Abstr.).

McDowell GH, Leenanuruksa HD, Nimsup P, Gooden JM, Van der Walt CJ, Smithard R. (1988). Short-term effects of exogenous growth hormone: effects on milk yield and composition, plasma hormones and metabolites. Aust. J. Biol. Sci. 41:279.

McGuffy RK, Basson RP, Spike TE (1991). Lactation response and body composition of cows receiving somatotrpin and three ratios of forage to concentrate. J. Dairy Sci. 74:3095.

McGuire MA, Bauman DE, Miller MA, Hartnell GF (1992). Responses of somatomedins (IGF-I and IGF-II) in lactating cows to variations in dietary energy and protein and treatrment with recombinant n-Methionyl bovine somatotropin. J. Nutr. 122:128.

Mellado M, Antonio-Chirino E, Meza-Herrera C, Veliz FG, Arevalo JR, Mellado J, Santiago A (2011). Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin. J Dairy Sci. 94:4524-4530.

Moallem U, Folman Y, Sklan D (2000). Effects of sometribove and dietary calcium soaps of fatty acids in early lactation on milk production, dry matter intake, and energy balance of high-yielding dairy cows. J. Dairy Sci. 83:2085.

Molento CF, Block E, Cue RI, Petitclere D (2002). Effects of insulin, recombinant bovine somatotropin, and their interaction on insulin-like growth factor-I and milk protein production in dairy cows. J. Dairy Sci. 85(4):738-747.

Moreira F, Badinga L, Burnley C, Thatcher WW (2002). Bovine somatotropin increases embryonic development in superovulated cows and improves post-transfer pregnancy rates when given to lactating recipient cows. Theriogenology 57, 1371-1387.

Nielsen MO (1988). Effect of recombinantly derived bovine somatotropin on mammary gland synthetic capacity in lactating goats. J. Anim. Physiol. 59A:263-269.

Nour El-Din ANM, El-Zarkouny SZ, Ghobashy H, Abdel-Gawad EI and Kesler DJ (2009). Growth, haematological and biochemical responses of growing lambs injected with growth hormone and supplemented with calcium soaps of fatty acids. South Afri. J. Anim. Sci. 2009, 39:65-68.

Nytes AJ, Combs DK, Shook GE, Shaver RD, Cleale RM (1990). response to recombinant bovine somatotropin (rbST) in dairy cows with different genetic merit for milk production. J. Dairy Sci. 73:784.

Ocampo LC, Morales M, Basurto HC, Auro AA (1995):Effect of somatotropin on milk production of cross-bred dairy cows in the tropics. Vet. Mexico 26:137.

Oldenbroek JK, Garssen GJ (1988). Effect of treatment of bST on milk production, feed intake and metabolism of dairy cows of three different breeds. Onderzoek bovine somatotropin. 23:15.

Oldenbroek JK, Garssen GJ (1990). Effect of bovine somatotropin on milk production and metabolism of dairy cows during three successive years in trials at the Dutch Cattle Breeding Research Institute. Tijdschrift voor Die rgneeskunde. 115:613.

Pell AN, Tsang DS, Howlett BA, Huyler MT, Messerole VK, Samules WA, Hartnell GF, Hintz RL (1992). Nutrition, feeding and calves-effects of a prolonged-release formulation of sometribove (n-methionylen bovine somatotropin) on Jersey cows. J. Dairy Sci. 5:3416-3431.

Phillips CJC (1996). Progress in dairy science. Pages 59-85. In: The effect of bovine somatotrophin on dairy production, cow health and economics. Cab International. Wallingford, Oxon, UK.

Phipps RH, Adriaens F, Hard DL, de Kerchove G, Weller RF, Kerchove De G (1996). Use of bovine somatotropin for milk production: The effect of time of treatment initiation on milk production of Holstein/-

Phipps RH, Madakadze C, Mutsvangwa T, Hard DL, Kerchove G (1991). Use of bovine somatotropin in the tropics: The effect of sometribove on milk production of *Bos indicus*, dairy crossbred and *Bos Taurus* cows in Zimbabwe. J. Agric Sci. 117:257.

Phipps RH, Weller RF, Craven N, Peel CJ (1990). Use of prolonged-release bovine somatotropin for milk production in British Friesian dairy cows. 1. Effect on intake, milk production and feed efficiency in two conscutive lacatations of treatment. J. Agric Sci. 115:95.

Friesian dairy cows placed on a high-forage, low concentrate feeding system. Grass and Forage Sci. 51:111.

Prosser CG, Fleet IR, Corps AN, Froesch ER, Heap RB (1990). Increase in milk secretion and mammary blood flow by intra-arterial infusion by insulin-like growth factor-I into the mammary gland of the goat. J. Endocrinol. 126:437.

Puchala R, Prieto I, Banskalieva V, Goetsch AL, Lachica M, Sahlu T (2001). Effects of bovine somatotropin and thyroid hormone status on hormone levels, bod weight gain and mohair fiber growth of Angora goats. J. Anim. Sci. 79:2913.

Pulina G, A Nudda, Pietro N, Macciotta P, Battacone G, Rassu SPG, and Cannas A (2007). Non-nutritional factors affecting lactation persistency in dairy ewes: a review. Ital. J. Anim. Sci. 6:115-141.

Qudus M A, Ahmad N, Javed K, Abdullah M, Jabbar M A, Omer M O, Iqbal Z M, and Ahmad I (2013). Effect of recombinant bovine somatotropin on milk production and composition of lactating Beetal goats. The J. Anim. Plant Sci. 23:26-30.

Rajamahendran R, Desbottes S, Shelford JA, Peterson RG, Kennelly JJ (1989). Effect of recombinant bovine somatotropin (rbST) on milk production and reproduction performance of dairy cows. J. Dairy Sci. 72; Suppl. 1:444.

Requena R, Balasch S, Peris C, Rodríguez M and Fernández N (2010). Dose response of lactating dairy ewes during suckling and milking to bovine somatotropin. J. Anim. Sci. 88:3136-3144.

Sallam SMA, Nasser MEA, Yousef MI (2005). Effect of recombinant bovine somatotropin administration on milk production, composition and some hemato-biochemical parameters of lactating goats. Egyptian J. Anim. Prod. 42(1):33-46.

Santos JEP, Huber JT, Theurer CB, Nussio LG, Nussio CB, Tarazon M, Lima-Filho RO (1999). Performance and nutrional digestibility by dairy cows treated with bovine somatotropin and fed diets with steam-flaked sorghum or steam-rolled corn during early lactation. J. Dairy Sci. 82:404-.

Schwab W, Kerchgessner M (1990). Recombinant bovine somatotropin (bGH) on nutrient digestibilty of dairy cows. 1. Influence of bGH on energy and protein metabolism of dairy cows at different stages of lactation. J. Anim. Physiol. and Nutr. 64:84.

Sechen SJ, Bauman DE, Tyrrell HF, Reynolds P.J. (1989a). Effect of somatotropin on kinetics on nonestrified fatty acids and partition of energy, carbon, nitrogen in lactating dairy cows J. Dairy Sci. 72:59.

Sechen SJ, McCutcheon SN, Bauman D.E. (1989b). Response to metabolic challenges in early lactation dairy cows during treatment with bovine somatotropin. Domest. Anim. Endocrinol. 6:141.

Settivarin RS, Spain JN, Ellersieck MR, Byatt JC, Collier RJ, Spires DE (2007). Relationship of thermal status to productivity in heat-stressed dairy cows given recombinant bovine somatotropin. J. Dairy Sci. 90:1265-1280.

Sharma BK, Vanderhaar MJ, Ames NK (1994). Expression of insulin-like growth factor-I in cows at different stages of lactation and in late lactation cows treated with somatotropin. J. Dairy Sci. 77:2232.

Smits NGE, Bremer MGEG, Ludwig SKJ, Nielen MWF (2012). Development of a flow cytometric immunoassay for recombinant bovine somatotropin-induced antibodies in serum of dairy cows. Drug Test Analysis 4:362–367.

Soderholm CG, Otterby DE, Linn JG, Ehle FR, Wheaton JE, Hansen WP, Annexstad RJ (1988). Effects of recombinant bovine somatotropin on milk production, body composition and physiological parameters. J. Dairy Sci. 71:355.

Spicer LJ, Stewart RE (1996). Interaction among bovine somatotropin, insulin an gonadotropins on steroid production by bovine granulosa and thecal cells. J.Dairy Sci. 79:813.

Staples CR, Head HH, Darden DE (1988). Short-term administration of bovine somatotropin to lactating dairy cows in a subtropical environment. J. Dairy Sci. 71:3274.

Stelwagen K, Grieve DG, Walton JS, Ball JL, McBride BW (1993). Effect of prepartum bovine somatotropin in primigravid ewes on mammogenesis, milk production and hormone concentrations. J. Dairy Sci. 76:992.

Thammacharoen S, Komolvanich S, Chanpongsang S, Chaiyabutr N (2011). Respiratory hypocapnia at different stages of lactation during long-term exogenous bovine somatotropin in crossbred Holstein cattle in the tropic. Thai J. Vet. Med. 4:245-250.

Vanderkool WK, Vandhaar MJ, Sharma BK, Binelli M, Tucker HA, Akers RM, Moseley WM (1995). Comparison of growth hormone-releasing factor and somatotropin: The somatotropic axis in lactating primiparous cows. J. Dairy Sci. 78:2140.

Weber WJ, Wallace CR, Hansen LB, Chester-Jones H, Crooker BA (2007). Effects of genetic selection for milk yield on somatotropin, Insulin-like growth factor-I and placental lactogen in Holstein cows. J. Dairy Sci. 90:3314.

Weller RF, Phipps RH, Craven N, Peel CJ (1990). Use of a prolonged-release bovine somatotrophin for milk production in British Friesian dairy cows. J. Agric. Sci. 115:105.

West JW, Bondari K, Johnson Jr. JC (1990). Effect of bovine somatotropin on milk yield and composition, body weight and condition score of Holstien and Jersey cows. J. Dairy Sci. 73:1062.

White TC, Madsen DS, Hintz RL, Sorbert RH, Colier RJ, Hard DL, Hartnell GF, Samules WA, Kerchove G, Adrianens F, Craven N, Bauman DE, Bertrand G, Bruneau PH, Gravert GO, Head HH, Huber JT, Lamb RC, Palmer C, Pell AN, Phipps R, Weller R, Riva G, Rijpkema Y, Skarda J, Vedeau F, Wollny C. (1994). Clinical mastitis in cows treated with sometribove (recombinant bovine somatotropin) and its relationship to milk yield. J. Dairy Sci. 77:2249.

Windsryg MD, Arambel MJ, Kent BA, Walters JL (1991). Effect of sometribove on rumen fermentation, rate of passage, digestibility, and milk production responses in dairy cows. J. Dairy Sci. 74:3518.

Zhao X, Burton JH, McBride BW (1992). Lactation, health and reproduction of dairy cows receiving daily injections of a sustained-release somatotropin. J. Dairy Sci. 75:3122.

Zinn S, Kazmer GW, Paquin-Platts DD (1993). Milk yield and composition response to a sustained-release formulation of bovine somatotropin in lactating dairy cows. J. Dairy Sci. 76 (Suppl.1):241 (Abst.).

Zoa-Mboe A, Head HH, Bachman KC, Baccari Jr F, CJ Wilcox (1989). Effects of bovine somatotropin on milk yield and composition, dry matter intake, and some physiological functions of Holstein cows during heat stress. J. Dairy Sci. 72:907-916.

academicJournals

Vol. 8(1), pp. 15-22, January 2014
DOI: 10.5897/JCAB2013.0399
ISSN 1996-0867 ©2014 Academic Journals
http://www.academicjournals.org/JCAB

Journal of Cell and Animal Biology

Full Length Research Paper

Increase of *Ace-1* resistance allele in the field population of *Anopheles gambiae* following a large scale indoor residual spraying (IRS) implementation using bendiocarb in Atacora region in Benin, West Africa

Rock Aïkpon[1,2]*, Nazaire Aïzoun[1,2], Arthur Sovi[1,2], Olivier Oussou[1], Renaud Govoetchan[1,2], Virgile Gnanguenon[1,2], Frédéric Oké-Agbo[1], Razaki Ossè[1,2] and Martin Akogbéto[1,2]

[1]Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604 Cotonou, Bénin.
[2]Faculté des Sciences et Techniques, Université d'Abomey Calavi, Calavi, Bénin.

Accepted 17 December, 2013

The aim of this study was to evaluate the susceptibility of *Anopheles gambiae* mosquitoes to bendiocarb, before (2010) and after (2012) the implementation of indoor residual spraying (IRS) interventions and to report the evolution of *Ace-1^R* mutation frequency in Atacora region. Indoor collection was carried out through Morning 7 to 9 a. m in five districts (Kouandé, Natitingou, Matéri, Tanguiéta and Copargo) of the Atacora-Donga region before and after IRS. Anopheles larvae were also reared in each district before and after IRS and emerging adults were exposed to bendiocarb (0.1%) in susceptibility tests. Polymerase chain reaction (PCR) assays were run to determine the members of the *An. gambiae* complex, as well for insensitive acetylcholinesterase (AChE1) due to *Ace-1^R* mutation. This study showed that the mean *Ace-1* mutation frequency had increased significantly from 2010 to 2012 after two years of an IRS. Mortality data indicated that mosquitoes were susceptible in 2010 to bendiocarb 0.1%. By 2012, there was a drastic decline in the *An. gambiae* susceptibility to bendiocarb in treated districts. *An. gambiae s.s.* and *Anopheles coluzzi* were the two members of *An. gambiae* complex that were found in sympatry in the study area. *An. gambiae* was predominant in tested samples (92.50%). The *Ace-1^R* mutation was found in both *An. gambiae s.s.* and *An. coluzzi* with frequency of 7.33 and 7.35%, respectively. The high proportion of homozygous susceptible specimens that survived from the WHO bioassays suggests the implication of biochemical resistance mechanisms. These results are of prime importance in the effort to document multiple impacts of operational control programmes on mosquito vectors, and to conceive sustainable control strategies for future malaria control programmes.

Key words: *Anopheles gambiae Ace-1^R*, increasing, indoor residual spraying, Benin.

INTRODUCTION

In recent years, huge progress has been made in the control of malaria in sub-Saharan African countries (Ceesay et al., 2008; O'Meara et al., 2008). In Benin, this progress is based on integrated approach of malaria con-

trol through vector-control, early diagnosis and treatment plus prophylaxis during pregnancy.

Malaria vector control in Benin is based on two interventions: the long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), both of which have been shown to be effective throughout Africa (Kelly-Hope et al., 2008; Protopopoff et al., 2007). Recent data from Zanzibar showed that the scale-up of LLIN, IRS and Artemisinin combination therapy (ACT) combined reduced malaria-related burden at health facilities by over 75% within five years (Aregawi et al., 2011). Similar results were noted on Bioko Island and Equatorial Guinea, where the simultaneous use of IRS, LLINs and ACT resulted in a 90% drop in the presence of *P. falciparum* circumsporozoite antigen in *An. gambiae s. l*. after four years. During the same period, malaria parasitaemia in children under five years old fell from 42 to 18% and mortality decreased to 70% (O'Meara et al., 2010).

It is in this context that, in 2011 in Atacora region, the National Malaria Control Programe (NMCP) of Benin decided to implement malaria vector control interventions with two major directions: the universal access to LLINs and IRS. The insecticide chosen by the NMCP to implement IRS in Atacora was the carbamate, bendiocarb (Akogbéto et al., 2010). The LLINs distributed were World Health Organization-approved Olyset® brand nets, impregnated with permethrin. But the main problem of the use of impregnated materials is the development of resistance. In recent years, insecticide resistance has been shown to be widespread in West Africa (Elissa et al., 1993; Akogbéto et al., 1999; Chandre et al., 1999; Yadouléton et al., 2010), in East Africa (Stump et al., 2004), Central Africa (Etang et al., 2006) and South Africa (Hargreaves et al., 2000, 2003).

In addition, several studies have shown the increase of vector resistance after vector interventions using insecticides. For example, in Uganda where dichloro-diphenyl-trichloroethane (DDT) is used for IRS and deltamethrin-impregnated insecticide-treated bednets (ITNs) are the cornerstone of vector suppression efforts, a significant increase in L1014S frequencies was observed in *An. gambiae s. s* in three out five sites during 2001 to 2002 and 2004 to 2006 (Verhaeghen et al., 2010). This allele shows the *Kdr* resistant to DDT and pyrethroids.

In Ethiopia, L1014F allele frequencies in excess of 98% were found in *An. arabiensis* exposed to DDT, insecticide used for IRS and ITN control measures (Della Torre et al., 2001). In a follow-up study after DDT was discontinued in favor of deltamethrin, more than 96% of *An. arabiensis* vectors were determined to be homozygous and 3.6% were found to be heterozygous for the L1014F allele (Padonou et al., 2012). These studies demonstrate that where pyrethroids and DDT have been applied intensively as part of vector interventions, selection of *kdr* alleles soon follows. However, in a recent study on the impact of a large scale

IRS and ITN campaign in southern Benin, *kdr* frequencies were also found to have increased; although this was also true for areas in which no planned intervention was implemented, underscoring the effect of the agricultural and household insecticide use on resistance (WHO, 2011).

The monitoring of insecticide resistance in malaria vectors is of prime importance especially where vector control programmes are planned or already running, in order to assess potential selection effects of insecticidal compounds on vector populations, and to take appropriate measures such as switching to other classes of compounds.

The aim of this study was to evaluate the susceptibility of *An. gambiae* mosquitoes to bendiocarb, before (2010) and after (2012) the implementation of IRS-interventions and to report the evolution of *Ace-1*^{R} mutation frequency in Atacora region.

METHODOLOGY

Study area

The study was carried out in Atacora-Donga region located in North-west of Benin and includes five districts: Kouandé, Matéri, Natitingou, Tanguiéta and Copargo (Figure 1). The five districts covered 12,571 km^2 and had an estimated population of 479, 8 92 in 2012. Atacora-Donga region has a sub-equatorial type climate with one dry season (December-May) and only one rainy season (June to November). The annual mean rainfall is 1,300 mm and the mean monthly temperature ranges between 22 and 33°C. The region is irrigated by three major rivers: the Mekrou, the Pendjari and the Alibori. The major economic activity is agriculture and it is characterized by the production of cotton and millet where various classes of pesticides are used for pest control.

Indoor residual spraying (IRS) campaigns (2011 to 2012)

The product chosen by the National Malaria Control Programme (NMCP) to implement IRS in Atacora was the carbamate, bendiocarb (Akogbéto et al., 2010). The formulation was 80% WP. The target dosage was 0.4 g a.i./m^2. Only one round of IRS was carried out per year. The IRS operation was performed by volunteers chosen from the local community who were trained by the PMI IRS partner. Each round covered over 90% of the households in the target districts.

Copargo district which has similar characteristics to the treated districts (climate, agricultural practices, LLIN coverage) was selected as control and was not treared. Twenty km separate the control (untreated) villages from the treated districts.

Study design and mosquito collections

In 2010 before the IRS intervention, mosquitoes were collected from five districts (Kouandé, Matéri, Natitingou , Tanguiéta and Copargo) situated in the Atacora-Donga region during the rainy season from September to October 2010 (Figure 1). In 2012 after IRS, mosquitoes were collected in same districts from August to

*Corresponding author. E-mail: rockypremier@yahoo.fr.

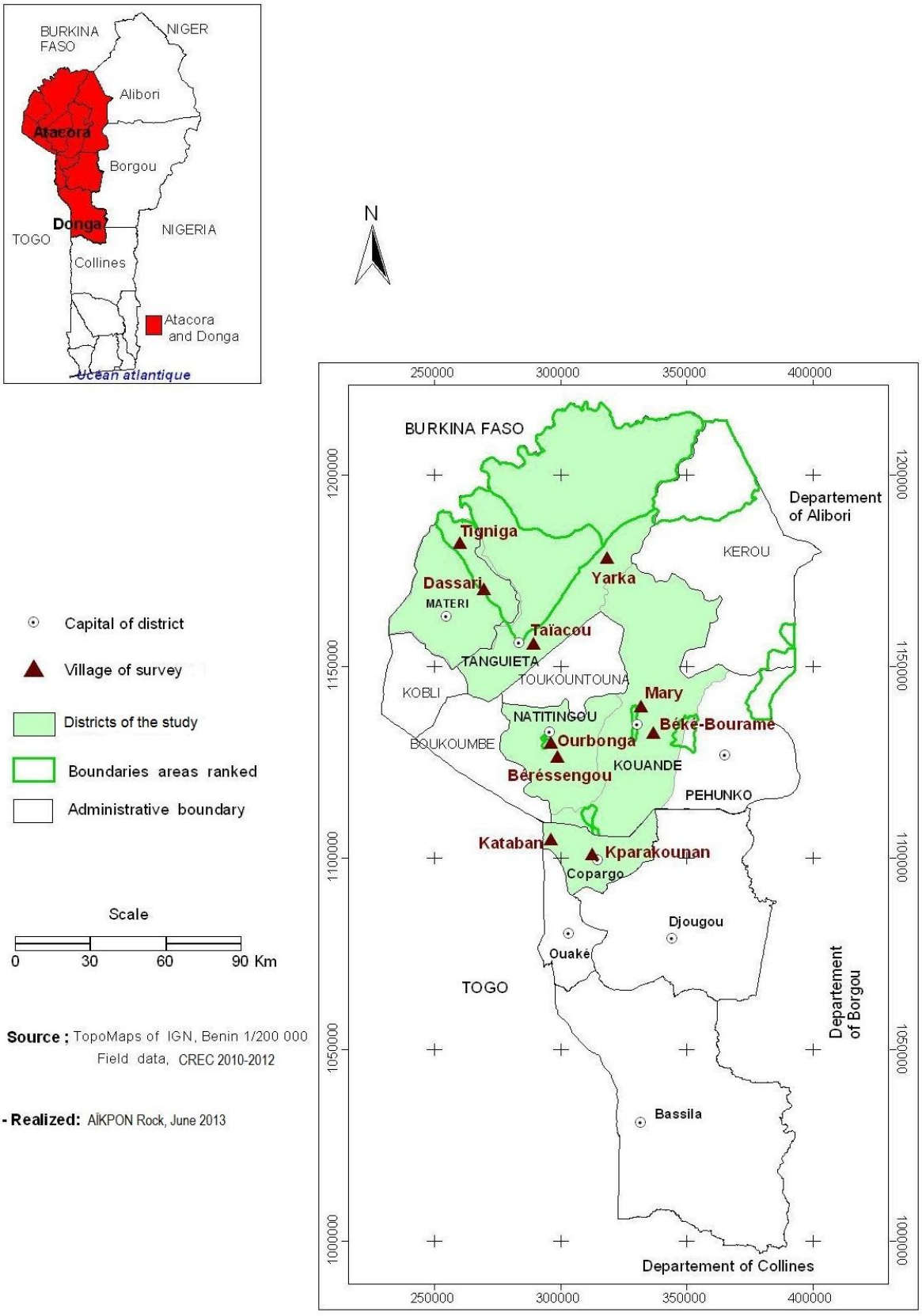

Figure 1. Map of Atacora Donga Departments showing the localities where anopheles mosquitoes were collected.

October (Figure 1).

Before the IRS intervention, mosquitoes resting in the house (indoor collection) were collected in Morning Spray Catches (MSC) from 7 to 9 a.m. In each district, two areas were selected: a central area and rural area. In each area, some ten houses were randomly selected for mosquito collection. Three sessions of mosquitoes collections were performed per month. MSC were performed using Rambo® and white canvas spread on the floor to collect knocked down mosquitoes. Knocked down mosquitoes falling on white bed sheets were kept separately and, kept in labeled tubes containing silica gel and frozen at -20°C before laboratory analysis.

In addition to MSC collection, *An. gambiae s. l*. larvae were collected in each locality. Larvae were collected from various natural breeding sites including ground pools, gutters, puddles and abandoned potholes. Water was collected using a plastic scoop and poured into small transparent plastic bowls. A strainer was used to sieve and pool together the third and fourth instar larvae in order to have sufficient adult emergence of the same physiological age. The mosquito larvae collected were transported in labelled plastic bottles to the laboratory of the Centre de Recherche Entomologique de Cotonou, Benin (CREC) where they were maintained at 28 ± 2°C and 72 ± 5% relative humidity.

In 2012, the same activities (larvae collection and MSC) were carried out from May to October. Four sessions of adult mosquito collections per month were carried out for three months. In each district, ten houses were selected per area for the mosquitoes collection.

Insecticide susceptibility tests

Adult mosquitoes from larvae collections were assayed using WHO discriminating dose with bendiocarb, 0.1%. Four batches of 25 unfed females, aged two to five days old, were exposed to the diagnostic doses on insecticide treated papers for 60 min at 27 ± 1°C and 80% relative humidity. The twenty-five females of *An. gambiae* were introduced into each tube and monitored at different time intervals (10, 15, 20, 30, 45, 60 min), the number “knocked-down” were recorded. After one hour exposure, mosquitoes were transferred into holding tubes and provided with cotton wool saturated with a 10% honey solution. Two batches exposed to untreated papers were used as control. A laboratory susceptible strain of *An. gambiae* Kisumu was used as a reference strain to compare the susceptibility levels of the field populations. Mortalities were recorded after 24 h and the susceptibility status of the population was graded according to the WHO protocol (WHO, 2011). Surviving mosquitoes from this bioassay were kept in eppendorf tubes containing silica gel and stored at −20°C before further molecular analysis.

Species identification

Adult mosquitoes were morphologically identified in the field and put in 96-well microplates with dessicant, and stored between -20 to -28°C in the laboratory before processing.

All mosquitoes collected by MSC and all live specimens from susceptibility test were subjected to the *An. gambiae* species specific PCR assays for species identification (Scott et al., 1993). Aliquots of DNA extracted from PCR positive specimens of *An. gambiae s.s.* were subjected to PCR assays for identification of *An. gambiae s. l* species (Favia et al., 1997).

Polymerase chain reaction (PCR) detection of *Ace.1^R* mutation

The PCR-RFLP diagnostic test was used to detect the presence of G119S mutation (*ace.1^R* gene) as described by Weill et al. 2004.

Data analysis

The resistance status of mosquito samples was evaluated according to the WHO criteria (WHO, 2011):

(i) Mortality rate is > 98%: the population is considered fully susceptible,
(ii) Mortality rates 90 - 98%: resistance is suspected in the population,
(iii) Mortality rates < 90%, the population is considered resistant.

To compare the status of bendiocarb resistance, Fisher's exact test was carried out to determine if there was any significant difference between mortality rates of populations of *An. gambiae s.s.* for 2010 to 2012. The treated districts and the control district in 2012 were also compared. The analyses were conducted using Statistica 6.0. Allelic frequencies of G119S mutation were analysed using the version 1.2 of Genepop (Raymond and Rousset, 1995). To assess if the mutation frequencies were identical across populations, the test of genotypic differentiation was performed (Goudet et al., 1996).

RESULTS

Anpheles gambiae s. l species and frequencies of the *Ace-1^R* mutation

All PCR analysis identifying *An. gambiae s. l.* species conducted in this study showed that all mosquitoes belonging to *An. gambiae s. l.* were *An. gambiae s.s.* A total of 935 *An. gambiae s. l* mosquitoes were identified for species and analyzed for the *Ace-1^R* mutation; results for *An gambiae* complex composition are shown in Table 1. *An. gambiae s.s.* and *An. coluzzii* occurred in sympatry in the study area. However, *An. gambiae s.s.* was predominant, representing 92.50% of the whole sample (n = 839). The *Ace-1^R* mutation was detected both in the homozygous and heterozygote state in *An. gambiae s.s.*, but only in the heterozygote state in *An. coluzzii*. No significant difference was seen between *Ace-1^R* mutation frequencies in *An. gambiae s.s.* (7.33%) and *An. coluzzii* (7.35%) (p = 0.2).

Ace-1^R genotype dynamic in adult *An. gambiae s. l.*

The MSC collection provided 107 *An. gambiae* in 2010, and 828 in 2012. The *Ace-1* allele frequencies *for An. gambiae* populations for the pre-intervention period (2010) and post-intervention period (2012) are shown in Table 2.

For the 2010 *Anopheles* collection, no homozygous resistant genotype was found, in any of the four districts where the mutation was detected, and only five heterozygous genotypes were found, giving frequency estimates ranging from 0 to 5.6%. The Ace-1 allelic frequency mutation gene was 1.9% in Kouandé, 0% in Tanguiéta, 5.6% in Natitingou, 2.9% in Matéri and 3.5 in Copargo.

In 2012, the *Ace-1* allelic frequency mutation gene had significantly increased in all of the treated districts. It was

Table 1. Distribution of acetylcholinesterase genotypes and frequency of *Ace-1*^{R} mutation in *Anopheles gambiae s.l* species in 2010 and 2012 collection.

District	*An. gambiae s.s.*				F(*Ace-1*)	*An. coluzzii*				F(*Ace-1*)
	n	RR	RS	SS		n	RR	RS	SS	
Kouandé	87	4	16	67	0.138[a]	2	0	0	2	0
Matéri	142	1	18	123	0.070[a]	2	0	0	2	0
Natitingou	90	2	20	68	0.33[a]	7	0	1	6	0.071
Tanguiéta	93	2	26	65	0.161[a]	9	0	2	7	0.111
Copargo	427	1	25	401	0.036[a]	48	0	7	41	0.073
Total	839	10	103	726	**0.073**	68	0	10	58	**0.074**

[a]Values sharing the same superscript letter were not significantly different at the 5% level for G119S mutation distribution.

Table 2. Comparison of *Ace-1* mutation allelic frequency for *An. gambiae* populations for pre-intervention period (2010) and post-intervention period (2012).

District	Year	N tested	*Ace-1 mutataion*				OR	CI-95%	P
			RR	RS	SS	F (*Ace-1*)			
Kouandé	2010	27	0	1	26	0.019[a]	1.00	-	-
	2012	62	4	15	43	0.185[b]	0.08	[0.01-0.63]	0.0015
Tanguiéta	2010	26	0	0	26	0.000[a]	1.00	-	-
	2012	76	2	28	46	0.211[b]	0.00	-	<0.0001
Natitingou	2010	9	0	1	8	0.056[a]	1.00	-	-
	2012	88	2	20	66	0.136[b]	0.34	[0.15-0.76]	0.0095
Matéri	2010	17	0	1	16	0.029[a]	1.00	-	-
	2012	127	1	17	109	0.075[b]	0.37	[0.05-2.89]	0.4859
Copargo	2010	28	0	2	26	0.036[a]	1.00		
(Control)	2012	475	1	32	442	0.036[a]	0.99	[0.23-4.26]	0.9317

[a]Values sharing a same superscript letter were not significantly different at the 5% level for G119S mutation distribution; OR: Odd ratio; CI: Confidence Interval.

18.5% in Kouandé, 21.1% in Tanguiéta, 13.6% in Natitingou, 7.5% in Matéri and 3.6% to Copargo.

Susceptibility to insecticides

In 2010, in all the districts, mortality rates ranged from 95 to 98% (Figure 2). From 2010 to 2012, there was a drastic decline in the *An. gambiae* susceptibility to bendiocarb in all treated districts. In fact, in 2012, the 24 h post-exposure mortality rate of *An. gambiae s. l* indicated resistance to bendiocarb from all treated localities with mortality rates ranging from 59 to 79% (Figure 2). When comparing susceptibility of *An. gambiae* to bendiocarb in 2010 and 2012, all of the districts except Copargo (the control) showed a significant decrease in mortality rate (Figure 2). Moreover, the comparison of the *An. gambiae* mortality rate to bendiocarb to Copargo between 2010 and 2012 showed no significant difference (p=0.24) (Figure 2). However, the slight decrease in the percentage of mortality in 2012 (90%), suggests a suspicion of resistance in Copargo.

Involvement of the *Ace-1*^{R} mutation in bendiocarb resistance in field populations of *An. gambae* from Atacora

To assess the involvement of the *Ace-1*^{R} allele in conferring bendiocarb resistance in *An. gambiae s.s.*, the *Ace-1*^{R} genotype was determined for mosquitoes not killed in WHO bioassay using bendiocarb in 2012 (Figure 3). Among bioassay survivors, all *Ace-1*^{R} genotypes (RR, RS and SS) were found. However, among the bioassay.

DISCUSSION

This study examined target site Insensitive Acetyl cholinesterase (*Ace-1*^{R}) in *An. gambiae* within an operational context in which IRS intervention was applied on a large scale in the department of Atacora in Benin. We identified a significant increase in the *Ace-1* allele frequency in all sites for which IRS was implemented.

In this study, two members of *An. gambiae* complex were found (*An. gambiae s.s.* and *An. coluzzii*) and their

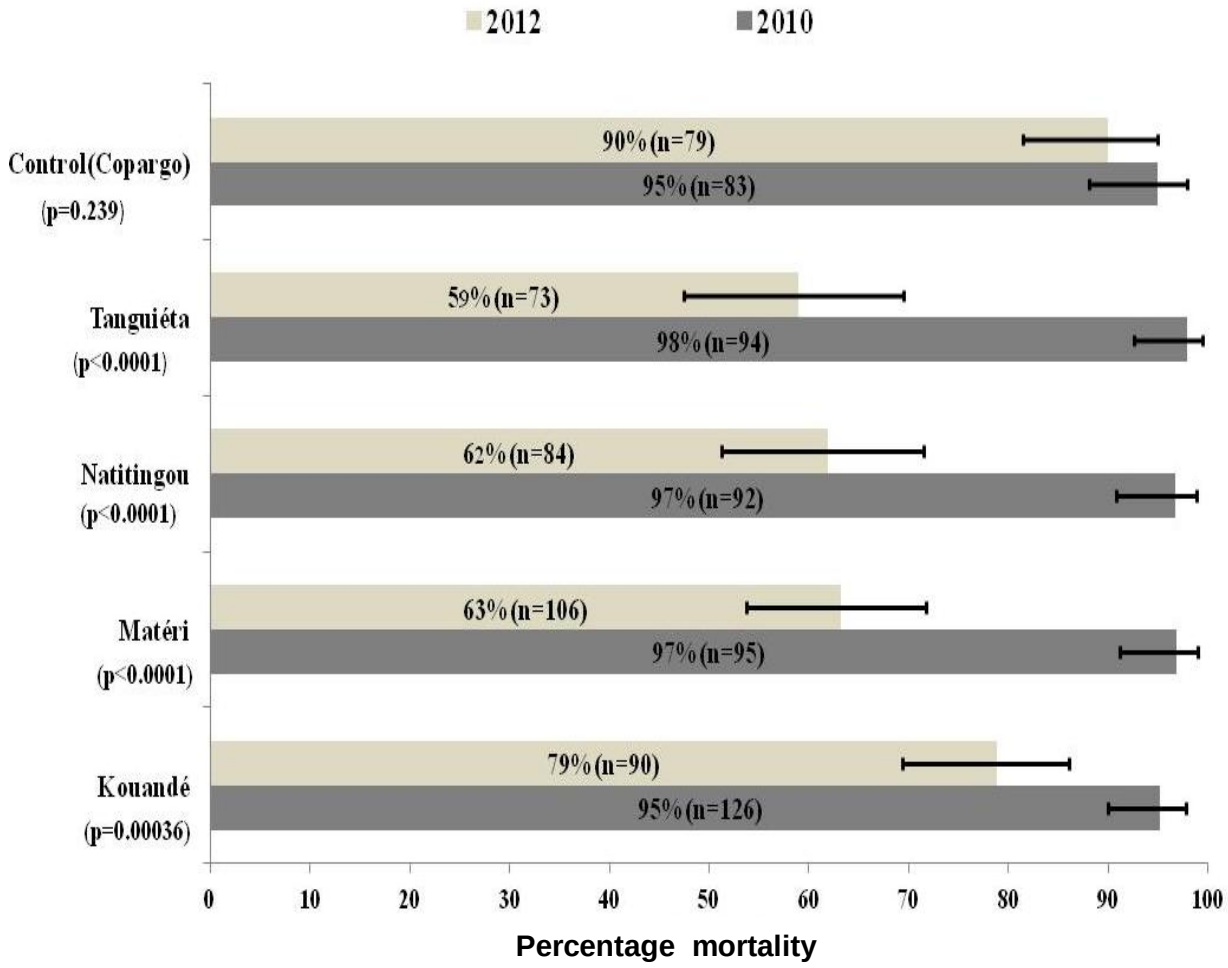

Figure 2. Bendiocarb mortality rate of *An. gambiae* populations for pre-intervention period (2010) and post-intervention period (2012).

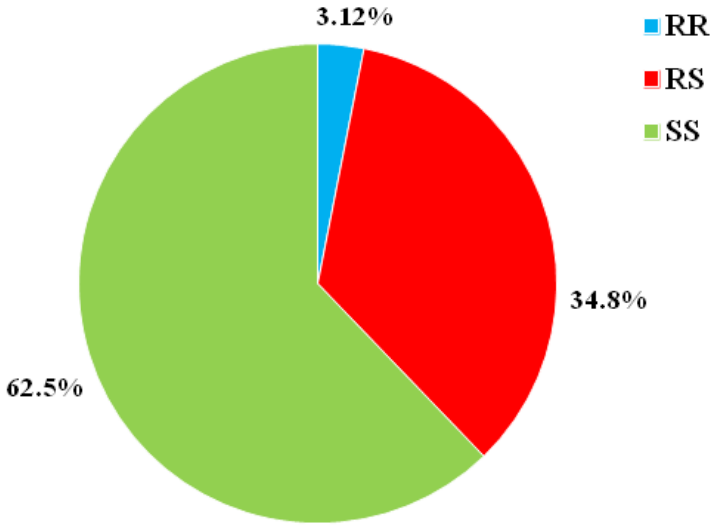

Figure 3. *Ace-1*^{R} genotypes frequencies found in *An. gambiae s.l* individuals not killed in WHO susceptibility test to bendiocarb.

the distribution agree with previous findings in Benin that reported both M and S forms with the predominance of S forms in a savannah areas (Djogbénou et al., 2008 and 2010). The presence of *Ace-1^R* mutation in *An. gambiae s.s.* and *An. coluzzii* has already been reported by Djogbénou (2010), and was suggested to result from introgression between forms. There is no significant difference of *Ace-1^R* mutation frequency between *An. gambiae s.s.* and *An. coluzzii*. The low number of homozygous resistant individuals might be related to high fitness cost of the *Ace-1^R* mutation, resulting in death of the homozygous resistant mosquitoes (Weill et al., 2004; Asidi et al., 2005; Djogbénou et al., 2010). The high number of heterozygous resistant RS is also in agreement with previous studies which showed that in areas where the resistant allele *Ace-1^R* is present, resistant mosquetoes will mainly be in the heterozygote state (RS) (Djogbénou et al., 2010; Ahoua-Alou et al., 2010).

From 2010 to 2012, the significant increase in the *Ace-1^R* frequency can be explained by the use of carbamates in public health that was greatly increased with implementation of IRS. Moreover, agricultural practices using insecticides may also be involved in the increase in *Ace-1^R* frequency and resistance to bendiocarb. Indeed, the evidence of an association between agricultural use of insecticides and the emergence of resistance in malaria vectors has been repeatedly reported. For example, in Côte d'Ivoire and Burkina Faso, N'Guessan (2003) reported that the level of vector resistance to pyrethroid insecticides increased during the cotton growing season.

In the current study, the WHO bioassays performed on *An. gambiae s.s.* from the study area in 2012 showed that the homozygous susceptible genotype (SS) is the most prevalent genotype among these survivors. The high proportion of homozygous susceptible specimens, which survived the WHO bioassays may suggest that the *Ace-1^R* mutation could not entirely explain the resistance of bendiocarb and highly suggests the implication of other resistance mechanisms such as metabolic detoxification. Further investigation is needed to evaluate the Biochemical mechanism that could be involved in the resistance of *An. gambiae* to bendiocarb in Atacora region.

Conclusion

This study demonstrated an increased frequency of *Ace-1^R* mutation in *An. gambiae* populations after the implementation of IRS in Atacora region (Benin). This increase in *Ace-1^R* frequency co-occured with an increased phenotypic resistance to bendiocarb. However, the increase in *Ace-1^R* mutation could not entirely explain the resistance to bendiocarb observed in 2012 and highly suggests the involvement of other resistance mechanisms such as metabolic detoxification. These results are of prime importance in our effort to document multiple effects of operational control programmes on mosquito vectors, and to conceive sustainable control strategies for the future. The documentation of the factors contributing to resistance selection within those populations is also highly important.

ACKNOWLEDGEMENTS

This work was financially supported by resident's Malaria Initiative (PMIP) through USAID. We thank the Ministry of Higher Education and Scientific Research (MESRS) and the team of CREC for their technical assistance during field work. We also thank the people of Atacora-Donga for their collaboration.

REFERENCES

Ahoua-Alou LP, Koffi AA, Adja MA, Tla E, Kouassi PK, Kone M, Chandre F (2010). Distribution of Ace-1R and resistance to carbamates and organophosphates in *Anopheles gambiae s.s.* populations from Côte d'Ivoire. Malar J. 9:167.

Akogbéto M, Yakoubou S (1999). Résistance des vecteurs du paludisme vis-à-vis des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires au Bénin, Afrique de l'Ouest. Bull Soc Path Exo. 92:123-130.

Akogbéto MC, Padonou GG, Gbénou D, Irish S, Yadouleton A (2010). Bendiocarb, a potential alternative against pyrethroid resistant *Anopheles gambiae* in Benin, West Africa. Malar J. 9:204.

Aregawi MW, Ali AS, Al-mafazy AW, Molteni F, Katikiti S, Warsame M, Njau RJA, Komatsu R, Korenromp E, Hosseini M, Low-Beer D, Bjorkman A, D'Alessandro U, Coosemans M, Otten M (2011). Reductions in malaria and anaemia case and death burden at hospitals following scale-up of malaria control in Zanzibar, 1999-2008. Malar J. 10:46.

Asidi AN, N'Guessan R, Koffi AA, Curtis CF, Hougard JM, Chandre F, Darriet F, Zaim M, Rowland MW (2005). Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae s.s.* and *Culex quinquefasciatus* mosquitoes. Malar J. 4:25.

Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJC, Sesay SSS, Abubakar I, Dunyo S, Sey O (2008). Changes in malaria indices between 1999 and 2007 in the Gambia: a retrospective analysis. Lancet. 372:1545-1554.

Chandre F, Manguin S, Brengues C, Dossou Yovo J, Darriet F, Diabate A, Carnevale P, Guillet P (1999). Current distribution of a pyrethroid resistance gene (*kdr*) in *Anopheles gambiae* complex from West Africa and further evidence for reproductive isolation of the Mopti form. Parasitologia. 41:319-322.

Della Torre A, Fanello C, Akogbeto M, Dossou-Yovo J, Favia G, Petrarca V, Coluzzi M (2001). Molecular evidence of incipient speciation within *Anopheles gambiae s.s*. in West Africa. Insect Mol Biol. 20:9-18.

Djogbénou L, Chandre F, Berthomieu A, Dabiré R, Koffi A, Alou H, Weill M (2008). Evidence of introgression of the Ace-1R mutation and of the Ace-1 duplication in West African *Anopheles gambiae s.s.* PLoS One. 3(5):2172.

Djogbénou L, Noel V, Agnew P (2010). Costs of insensitive acetylcholinesterase insecticide resistance for the malaria vector *Anopheles gambiae* homozygous for the G119S mutation. Malar J. 9:12.

Djogbénou L, Pasteur N, Bio-Bangana S, Baldet T, Irish SR, Akogbeto M, Weill M, Chandre F (2010). Malaria vectors in the Republic of Benin: Distribution of species and molecular forms of the *Anopheles gambiae* complex. Acta Trop. 114:116-122.

Elissa N, Mouchet J, Rivière F, Meunier JY, Yao K (1993). Resistance of *Anopheles gambiae s.s.* to pyrethroids in Côte d'Ivoire. Ann Soc Belge Med Trop. 73:291-294.

Etang J, Fondjo E, Chandre F, Morlais I, Brengues C, Nwane P, Chouaibou M, Ndjemai H, Simard F (2006). First report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. Am. J. Trop. Med. Hyg. 74:795-797.

Favia G, Della Torre A, Bagayoko M, Lanfrancotti, Sagnon NF, Toure Y, Coluzzi M (1997). Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. Insect Mol Biol. 6:377-383.

Goudet J, Raymond M, De Meeüs T, Rousset F (1996). Testing differentiation in diploid populations. *Genetics*. 144:1933-1940.

Hargreaves K, Hunt RH, Brooke BD, Mthembu J, Weeto MM, Awolola TS, Coetzee M (2003). *Anopheles arabiensis* and *An. quadriannulatus* resistance to DDT in South Africa. Med. Vet. Entomol. 17:417-422.

Hargreaves K, Koekemoer L, Brooke BD, Hunt RH, Mthembu J, Coetzee M (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med. Vet. Entomol. 14:181-189.

Kelly-Hope L, Ranson H, Hemingway J (2008). Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. Lancet Infect Dis. 8:387-389.

N'Guessan F, Darriet P, Guillet P, Carnevalle M, Lamizana T, Corbel V, Chandre F (2003). Resistance to carbosulfan in Anopheles gambiae base on reduced of acethylcholinesterase. Med. Vet. Entomol. 17:19-25.

O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CRJC, Marsh K (2008). Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet. 372:1555-1562.

O'Meara WP, Mangeni JN, Steketee R, Greenwood B (2010). Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect. Dis. 10:545-555.

Padonou GG, Sezonlin M, Ossé R, Aizoun N, Oké-Agbo F, Oussou O, Gbédjissi G, Akogbéto M (2012). Impact of three years of large scale indoor residual spraying (IRS) and insecticide treated nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae* s.l. in Benin. Parasit. Vectors. 5:72-82.

Protopopoff N, Van Herp M, Maes P, Reid T, Baza D, D'Alessandro U, Van Bortel W, Coosemans M (2007). Vector control in a malaria epidemic occurring within a complex emergency situation in Burundi: a case study. Malar J. 6:93.

Raymond M, Rousset F (1995). Genepop (version 1.2), population genetics software for exact tests and eucumenicism. J. Heredity. 86:248-249.

Scott JA, Brogdon WG, Collins FH (1993). Identification of single specimens of *Anopheles gambiae* complex by polymerase chain reaction. Am. J. Trop. Med. Hyg. 49:520-529.

Stump AD, Atieli FK, Vulule JM, Besansky NJ (2004). Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *Anopheles gambiae* in response to insecticide-treated bed net trials. Am. J. Trop. Med. Hyg. 70:591-596.

Verhaeghen K, Bortel WV, Roelants P, Okello PE, Talisuna A, Coosemans M (2010). Spatio-temporal patterns in kdr frequency in permethrin and DDT resistant *Anopheles gambiae s.s.* from Uganda. Am. J. Trop. Med. Hyg. 82:566-573.

Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond M (2004). The unique mutation in *Ace-1* giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol. Biol. 13:1-7.

WHO :*Recommandations de la consultation technique sur la lute contre les vecteurs du paludisme dans la Région africaine de l'OMS*. Brazaville: Congo/Rapport technique de l'OMS; 2011:2.

Yadouleton AW, Padonou G, Asidi A, Moiroux N, Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC (2010). Insecticide resistance status in *Anopheles gambiae* in southern Benin. Malar. J. 9:83.

UPCOMING CONFERENCES

BIT's 4th Annual World Congress of Molecular & Cell Biology (CMCB2014), Dalian, China, 25 Apr 2014

International Conference on Cellular and Molecular Biology, Copenhagen, Denmark, 12 Jun 2014

ICCMB 2014 : International Conference on Cellular and Molecular Biology

Conferences and Advert

February 2014
7th Asia-Pacific Organization for Cell Biology Congress, Biopolis, Singapore, 24 Feb 2014

March 2014
American College of Medical Genetics and Genomics (ACMG) Annual Clinical Genetics Meeting, Nashville, USA, 25 Mar 2014

April 2014
18th Annual International Conference on Research in Computational Molecular Biology, Pittsburgh, USA, 02 Apr 2014

Society for General Microbiology Annual Conference, Liverpool, UK, 14 Apr 2014

BIT's 4th Annual World Congress of Molecular & Cell Biology (CMCB2014), Dalian, China, 25 Apr 2014

May 2014
International Conference on Biochemistry and Molecular Biology, Vienna, Austria, 17 May 2014

International Conference on Molecular Biology, Biochemistry and Biotechnology, Tokyo, Japan, 29 May 2014

June 2014
16th International Conference on the Cell and Molecular Biology of Chlamydomonas, Pacific Grove, USA, 08 Jun 2014

Tissue Engineering and Regenerative Medicine International Society (TERMIS) EU 2014 Congress, Genova, Italy, 10 Jun 2014

International Conference on Cellular and Molecular Biology, Copenhagen, Denmark, 12 Jun 2014

11th Gordon Conference on Cell Biology of the Neuron, Waterville Valley, USA, 22 Jun 2014

EMBO Conference on Microbiology after the Genomics Revolution: Genomes 2014, Paris, France, 24 Jun 2014

Journal of Cell and Animal Biology

Related Journals Published by Academic Journals

- *International Journal of Genetics and Molecular Biology*
- *Journal of Microbiology and Antimicrobials*
- *International Journal of Biodiversity and Conservation*
- *Journal of Bacteriology Research*
- *Journal of Developmental Biology and Tissue Engineering*
- *Journal of Evolutionary Biology Research*

academicJournals