

## INFLUENCE OF HONEY BEE VENOM ON GROWTH PERFORMANCE OF ZARAIBI KIDS FROM WEANING TO SEXUAL PUBERTY

E.I. Khalifa<sup>1</sup>, Sarah Ibrahim Grawish<sup>2</sup>, A.A.A.I. Gomaa<sup>1</sup>, Ghania A.M.M<sup>2</sup>, A.A.A. Metwally<sup>2</sup> and T.M.M. Mahdy<sup>1</sup>

*1-Animal Production Research Institute (APRI), Agriculture Research Center, Dokki, Giza, Egypt, 2-Honey Bee Research Department- Plant Protection Research Institute- Agriculture Research Center*

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### SUMMARY

This study was conducted to evaluate the effect of bee venom (BV) injection on growth performance of Zaraibi kids up to sexual puberty. Twelve weaned kids (aged 90 days with average body weight  $10.50 \pm 0.54$  kg) were randomly divided into three equal groups ( $n=4$  kids / treatment). The 1<sup>st</sup> group of kids was fed on basal diet plus injected weekly with 1.0 ml of distilled water without BV and served as a control group (BV0). The 2<sup>nd</sup> and 3<sup>rd</sup> treated kids were fed on the same basal diet and injected weekly with 1.0 ml of distilled water added to 250 or 500 mg/ml of BV / kid, representing BV1 or BV2 experimental groups, respectively. All BV0, BV1 and BV2 kids were rearing to estimate growth rate, measurement of scrotal circumference, testes volume, testosterone level through sexual puberty phases and blood parameters. The results showed that groups BV1 or BV2 had significantly higher growth performance than BV0 kids. Both BV1 and BV2 kids had significantly ( $P<0.05$ ) higher measurement of scrotal circumference, testes volume and testosterone level through sexual puberty phases compared with BV0 kids. Also, blood parameters had improvement ( $P<0.05$ ) values for both BV1 or BV2 kids compared to BV0 kids. Furthermore, BV1 and BV2 kids showed a positive effect ( $P<0.05$ ) on antioxidant parameters while malondialdehyde (MDA) significantly decreased ( $P<0.05$ ) compared with BV0 kids. In conclusion, BV could be used to enhance growth performance, reducing the times of sexual puberty phases, improving immunological and ant oxidative responses in kids.

**Keywords:** Kids, productive performance, honey bee venom

### INTRODUCTION

Recently, using natural substances in feeding led to increase interest in human nutrition. Hence, among the various honey bee products of natural origin as pollen, propolis and royal jelly, and bee venom (BV) are mainly bee products known as apitoxin (Hegazi *et al.*, 2014). The BV produced by female bees' worker is a complex mixture of substances which has biological activity (Uddin *et al.*, 2016). In addition, BV has been used to relieve pain and to treat chronic inflammatory diseases and even neurologic disorders (Rady *et al.*, 2017). In this context, Mammadova and Topchiyeva (2017) reported that BV has melittin, abundant enzymes, proteins and peptides; also it has other smaller substances as amino acids, catecholamines, sugars and minerals.

The BV is a light-yellow liquid includes 88% water, and consists of a complex mixture of amines, enzymes, bioactive peptides, antimicrobial activity, with a pH ranging between 4.5 and 5.5 (Zhang *et al.*, 2018). Also, Pino-Angeles and Lazaridis (2018) reported that BV has immunity promoting properties. The melittin and apamin components in BV, have antiinflammatory, antifibrotic, immunomodulatory activity, also BV peptides had positive directly affect on cell membranes as reported by Zarrinnahad *et al.* (2018). Furthermore, Rabie *et al.* (2018a) reported that BV has proteins, peptides (melittin, apamine and

adolapine) and other molecular components (dopamine, histamine and norepinephrine). Confirmation was reported by Frangieh *et al.* (2019) who noticed that BV has many active components including phospholipase A2 (PLA2) enzymes, hyaluronidase amino acids and volatile compounds. Consequently, BV could be a feasible significant alternative to antimicrobial growth promoters to prevent the use of antibiotics that are regularly used in animal feeding (Al-Shammari *et al.*, 2017). As a result, BV can be used safely as a healthy supplement in rabbit industry (Rabie *et al.*, 2018b), it is a highly productive factor in the broiler (Kim *et al.*, (2019) and it has a positive action on the immune system which reflected in improved productive performance in male rabbits (El-Hanoun *et al.*, 2020). El-Seedi *et al.* (2020) found that BV can improved the productivity and health status of growing farm animals. Likewise, Elkomy *et al.* (2023) noticed improvement in final growing weight of rabbits up to 1974, 2089, 2160 and 2138g with 0, 2, 4 and 8 mg BV/ kg body weight, respectively. Most researches in that area studied the effeteness of BV improving growth in chickens and rabbits. However, there is a significant lack of reports that study the influence BV on productive performance of growing male Zaraibi kids up to sexual puberty.

### MATERIALS AND METHODS

In this current investigation, all experimental animals belonged to EL-Serw Research Station, Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation (MALR), Egypt. This study was performed from March to November 2023.

#### **Animals, housing environment, and experimental feeding:**

Twelve healthy and weaning males of Zaraibi kids aged 90-days, with an average body weight of  $10.50 \pm 0.54$  kg, were divided into three treatments ( $n=4$  males for each treatment).

The first group received a basal diet + 1.0 ml of

distilled water injected intramuscular weekly/ kid to form control group (BV0). The second and third experimental groups received same basal diet + 1.0 ml of distilled water containing either 250 or 500 mg of (BV), injected intramuscular weekly/ kid, to form BV1 or BV2 groups, respectively.

All BV0, BV1 and BV2 kids of each group were housed separately from weaning to sexual maturity under similar natural environmental condition. The experimental diet covered all the kids' nutritional requirements according to NRC (2007). The chemical analysis of the diet was reported according to AOAC (2007) recommendations, as shown in Table (1).

**Table 1. Chemical analysis and ingredients feeding values of basal experimental diet (on DM basis)**

| Chemical composition (%)              | Basal experimental diets |       |       |
|---------------------------------------|--------------------------|-------|-------|
|                                       | CFM*                     | BH    | RS    |
| Organic matter (OM)                   | 87.60                    | 84.03 | 84.95 |
| Crude protein (CP)                    | 14.42                    | 14.90 | 3.81  |
| Ether extract (EE)                    | 2.44                     | 1.03  | 1.62  |
| Crude fiber (CF)                      | 7.33                     | 30.83 | 39.91 |
| Nitrogen free extract (NFE)           | 63.41                    | 37.27 | 39.61 |
| Ash                                   | 12.40                    | 15.97 | 15.05 |
| <b>Ingredients feeding values**</b>   |                          |       |       |
| Total digestible nutrients (TDN)      | 62.76                    | 65.25 | 54.49 |
| Digestible crude protein (DCP)        | 10.29                    | 10.75 | 0.11  |
| Digestible energy (DE) M cal/kg DM    | 2.77                     | 2.88  | 2.40  |
| Metabolizable energy (ME) M cal/kg DM | 2.35                     | 2.46  | 1.97  |
| Net energy (NE) M cal/kg DM           | 1.42                     | 1.47  | 1.23  |

\*The CFM consisted of 26 % undecortecated cotton meal, 40% yellow corn, 27% wheat bran, 3.5% molasses, 2% limestone, 1 % common salt and 0.5 % minerals mixture, \*\* Ingredients feeding values as total digestible nutrients (TDN) =  $129.39 - 0.9419 (CF + NFE)$ , digestible crude protein (DCP) =  $0.9596 (CP) - 3.55$ , digestible energy (DE) M cal/kg DM =  $0.04409 (TDN \%)$ , metabolizable energy (ME) =  $1.01(DE) - 0.45$  and net energy (NE) =  $0.0245 (TDN \%) - 0.12$  was calculated according to NRC (2007).

#### **Collection of data:**

##### **Growth performance of weaning males:**

The productive performances, such as final body weight (FBW), daily weight gain (DWG), total feed intake (TFI), feed conversion ratio (FCR), performance index (PI) and productive efficiency factor (PEF) were determined according to Mahdy et al. (2023).

##### **Measurement of scrotal circumference, testes volume and testosterone level through sexual puberty phases:**

The three sexual pubertal phases of kids were studied by using an estrus goat to service up to 30 minutes for mating described by Behery et al. (2015). The kids' scrotal circumference was assayed by Mahdy et al. (2023). The testes volume ( $\text{cm}^3$ ) =  $0.015409 \times SC^3$  was determined according to Entwistle (1992). Blood Testosterone level was determined at each phase of puberty using kits produced by a German IBL Company; with catalogs No RE52151 and the ELISA Plate Reader (Biotek ELX808, USA made).

##### **Blood parameters:**

In the morning of test day, nine blood samples (three kids / treatment) were collected from jugular veins before feeding. The serum was obtained by centrifugation of the blood samples at 3500 rpm for 20 min. and stored at  $-20^\circ\text{C}$  for later analysis to determine blood parameters as follows:

##### **Biochemical blood components:**

Total protein, albumin, globulin, total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides were measured using commercial assay kit obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Also, the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed spectrophotometrically according to the manufacturer's protocol using commercial kits (Bio-diagnostic, Giza, Egypt).

##### **Blood antioxidant status and immune response:**

Total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase

enzyme activities (CAT) were determined using commercial kits (Shmadzu, Kyoto, Japan) according to the manufacturer's instructions. The immune response was assayed as immunoglobulins G (IgG) and immunoglobulins M (IgM) using commercial kits # CSB-E06950RB.

**Statistical Analysis:**

The values of measured and calculated parameters were expressed as the mean values ± standard error "SE". Statistical analysis was carried out using a one-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test by using the SPSS/PC computer program (SPSS Statistics version 2020). Different letters mean a significant difference at  $P < 0.05$  in the same row. Correlation coefficients between BV and different studied traits were calculated by Pearson SPSS/PC computer program.

The test in a completely randomized design as the following model:-

$$Y_{ik} = \mu + T_i + e_{ik}$$

$Y_{ik}$  = an observation.

$\mu$  = the overall mean.

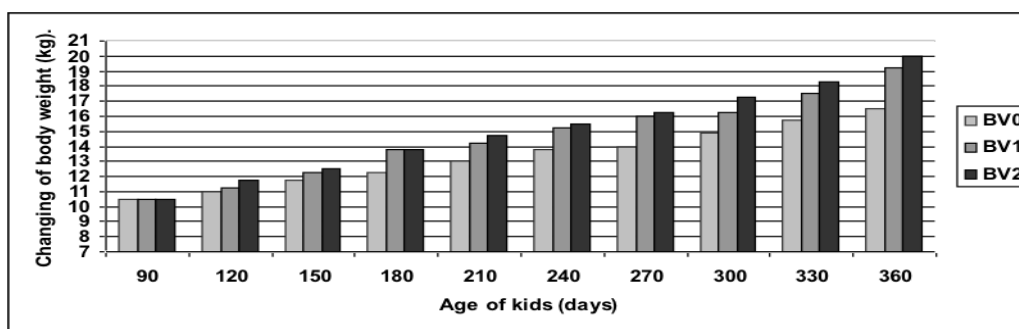
$T_i$  = the fixed effect of  $i^{th}$  where  $I = 1, 2, 3$  treatments ( $1 = BV0, 2 = BV1$  and  $3 = BV2$ ).

$e_{ik}$  = residual error.

**RESULTS**

**Growth performance up to final body weight (FBW) in bee venom treatments as BV0, BV1 and BV2 kids:**

Obtained data supported that injection BV to kids resulted in a significant increase ( $P > 0.05$ ) in FBW compared to the control group, as shown in Figure (1). At 360 days of age, the BV0, BV1 and BV2 kids showed no significant differences in FBW; values reached  $16.50 \pm 1.44$ ,  $19.25 \pm 1.11$  and  $20.00 \pm 1.47$  kg, for the three groups respectively. Furthermore, obtained results showed that the BV2 kids had higher ( $P > 0.05$ ) values in FBW than the BV1 kids.



**Fig. 1.** The changing of body weight from 90 to 360 days of age for bee venom treatments as BV0, BV1 and BV2 kids.

**Measurement of FBW, DWG, TFI, FCR, PI, metabolic weight (MW) and PEF in bee venom treatments as BV0, BV1 and BV2 kids:**

The results explained in Table (2) indicated that there were no significant differences in FBW, TFI and MW among BV0, BV1 and BV2 kids, but BV2 kids' were higher ( $P > 0.05$ ) compared to BV1 kids.

Kids in both BV1 and BV2 kids had significantly higher ( $P < 0.05$ ) DWG, PI and PEF compared to BV0 kids. Also, results supported that BV1 or BV2 kids compared with BV0 kids, has significantly ( $P < 0.05$ ) improved FCR value.

**Table 2.** Measurement of FBW, DWG, TFI, FCR, PI, PEF and MW in bee venom treatments as BV0, BV1 and BV2 kids

| Items                       | Bee venom treatments       |                            |                            |
|-----------------------------|----------------------------|----------------------------|----------------------------|
|                             | BV0                        | BV1                        | BV2                        |
| Initial body weight, kg     | 10.50±0.65                 | 10.25±1.04                 | 10.50±1.32                 |
| FBW, kg                     | 16.50±0.1.44               | 19.25±1.11                 | 20.00±1.47                 |
| TFI during trail periods, g | 535.75±28.62               | 555.88±25.61               | 541.50±32.63               |
| DWG, g                      | 22.22 <sup>b</sup> ±3.02   | 32.41 <sup>a</sup> ±2.33   | 35.19 <sup>a</sup> ±2.39   |
| FCR, %                      | 25.05 <sup>a</sup> ±2.38   | 17.46 <sup>b</sup> ±1.62   | 15.52 <sup>b</sup> ±1.01   |
| PI, %                       | 65.87 <sup>b</sup> ±11.70  | 110.25 <sup>a</sup> ±10.83 | 128.87 <sup>a</sup> ±10.05 |
| MW                          | 9.00±0.49                  | 9.49±0.54                  | 9.61±0.72                  |
| PEF, %                      | 145.45 <sup>b</sup> ±32.17 | 213.90 <sup>a</sup> ±29.77 | 242.42 <sup>a</sup> ±27.63 |

Means in the same row within each classification bearing different letters are significantly different ( $P < 0.05$ ).

Final body weight (FBW), daily weight gain (DWG), total feed intake (TFI), feed conversion ratio (FCR), performance index (PI) and productive efficiency factor (PEF).

*The measurements of scrotal circumference (SC), testes volume and testosterone level in bee venom*

*treatments as BV0, BV1 and BV2 kids through sexual puberty phases as following: -*

### Scrotal circumference (SC):

From Figure (2), it could be concluded that treatment of growing kids with either BV1 or BV2 kids could improve measurements of SC comparing with BV0 group for the experimental period. The SC was gradually increased in BV0, BV1 and BV2 kids up to 360 days of age but the differences were

insignificant. Then, BV1 (22.00 cm) and BV2 (22.75 cm) kids in additional measurement, compared SC of 21.25 cm for BV0 kids. However, it should be noted that there were no significant differences between BV1 and BV2 in SC measurements throughout the experiment periods.

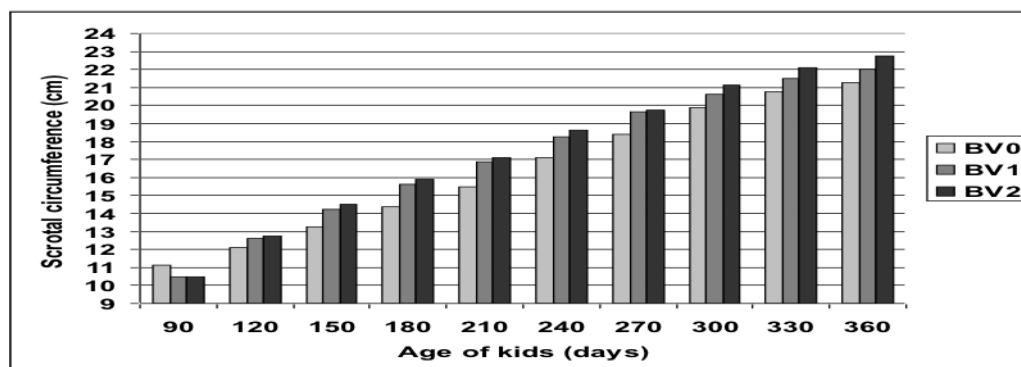


Fig. 2. The measurement of scrotal circumference from 90 to 360 days of age for bee venom treatments as BV0, BV1 and BV2 kids.

**Sexual puberty phases, testes volume and testosterone concentration of kids in bee venom treatments as BV0, BV1 and BV2 kids: It is apparent from Table (3) that the BV1 and BV2 kids reached to 1st phase of sexual puberty faster ( $P<0.05$ ) than BV0 kids.**

In the 2<sup>nd</sup> phase of sexual puberty the age, the most ( $P<0.05$ ) testicular volume (TV) and testosterone concentration (TC) values were evident

in BV1 and BV2 kids compared with BV0 kids. Data observed that age, body weight, TV and TC had more significant ( $P<0.05$ ) parameters in BV1 and BV2 kids than BV0 kids during 3<sup>rd</sup> phase of sexual puberty. Finally, the result indicated that there were no significant differences between BV1 and BV2 kids in testes volume and testosterone concentration, but BV2 group showed superiority ( $P>0.05$ ) through the three phases of sexual puberty.

Table 3. Sexual puberty phases, testes volume and testosterone concentration in bee venom treatments as BV0, BV1 and BV2 kids

| Items  | Bee venom treatments      |                            |                           |
|--|---------------------------|----------------------------|---------------------------|
|  | BV0                       | BV1                        | BV2                       |
| <b>1<sup>st</sup> phase: mating with desire trembling without penis exposure</b>       |                           |                            |                           |
| Age, days  | 186.75 <sup>a</sup> ±5.51 | 164.50 <sup>b</sup> ±1.31  | 161.51 <sup>b</sup> ±2.40 |
| Body weight, kg  | 12.33 <sup>b</sup> ±0.24  | 13.37 <sup>a</sup> ±0.23   | 13.50 <sup>a</sup> ±0.41  |
| Scrotal circumference, Cm  | 14.38 <sup>b</sup> ±0.43  | 15.00 <sup>ab</sup> ±0.29  | 15.5 <sup>a</sup> ±0.20   |
| Testicular volume, Cm <sup>3</sup>   | 46.14 <sup>b</sup> ±4.18  | 52.48 <sup>ab</sup> ±3.00  | 57.47 <sup>a</sup> ±2.27  |
| Testosterone concentration, ng/ml  | 0.67 <sup>b</sup> ±0.04   | 0.85 <sup>ab</sup> ±0.03   | 1.01 <sup>a</sup> ±0.23   |
| <b>2<sup>nd</sup> phase: mating with penis exposure</b>                                |                           |                            |                           |
| Age, days  | 211.00 <sup>a</sup> ±8.48 | 187.50 <sup>b</sup> ±4.74  | 175.00 <sup>b</sup> ±2.04 |
| Body weight, kg  | 13.63±0.31                | 14.13±0.24                 | 14.25±0.32                |
| Scrotal circumference, Cm  | 15.12±0.63                | 16.13±0.38                 | 16.25±0.32                |
| Testicular volume, Cm <sup>3</sup>   | 54.13 <sup>b</sup> ±6.49  | 64.92 <sup>a</sup> ±4.30   | 66.36 <sup>a</sup> ±3.94  |
| Testosterone concentration, ng/ml  | 0.78 <sup>b</sup> ±0.03   | 1.31 <sup>a</sup> ±0.21    | 1.50 <sup>a</sup> ±0.15   |
| <b>3<sup>rd</sup> phase: mating with first semen ejaculate containing motile sperm</b> |                           |                            |                           |
| Age, days  | 222.50 <sup>a</sup> ±8.54 | 206.25 <sup>ab</sup> ±8.98 | 190.00 <sup>b</sup> ±4.08 |
| Body weight, kg  | 14.12 <sup>b</sup> ±0.23  | 14.88 <sup>ab</sup> ±0.23  | 15.00 <sup>a</sup> ±0.29  |
| Scrotal circumference, Cm  | 16.13±0.62                | 17.00±0.20                 | 17.13±0.24                |
| Testicular volume, Cm <sup>3</sup>   | 65.47 <sup>b</sup> ±7.38  | 75.80 <sup>a</sup> ±2.73   | 77.52 <sup>a</sup> ±3.21  |
| Testosterone concentration, ng/ml  | 0.92 <sup>b</sup> ±0.03   | 1.75 <sup>a</sup> ±0.13    | 1.93 <sup>a</sup> ±0.14   |

Means in the same row within each classification bearing different letters are significantly different ( $P<0.05$ ).

**Biochemical blood components in bee venom treatments as BV0, BV1 and BV2 kids:**

Data clarified in Table 3, indicates that BV1 and

BV2 kids had higher ( $P<0.05$ ) total protein and globulin levels than BV0 kids. Within the same context, the albumin level did not differ among BV0,

BV1 and BV2 kids. The liver function enzymes had lower ( $P<0.05$ ) activities in both BV1 and BV2 kids compared to values obtained for the BV0 kids, which showed an adverse effect on liver enzymes as AST and ALT concentrations. Both total cholesterol and

triglyceride were decreased ( $P<0.05$ ) in BV1 and BV2 kids compared to the BV0 kids. Both BV1 and BV2 kids had reduction ( $P<0.05$ ) in LDL, in contrast to HDL concentrations in the BV0 kids.

**Table 4. Blood components in bee venom treatments as BV0, BV1 and BV2 kids**

| Items               | Bee venom treatments      |                           |                           |
|---------------------|---------------------------|---------------------------|---------------------------|
|                     | BV0                       | BV1                       | BV2                       |
| Total protein, g/dL | 5.17 <sup>b</sup> ±0.13   | 6.81 <sup>a</sup> ±0.15   | 6.90 <sup>a</sup> ±0.12   |
| Albumin, g/dL       | 4.20±0.17                 | 3.67±0.19                 | 3.63±0.18                 |
| Globulin, g/dL      | 1.07 <sup>b</sup> ±0.9    | 3.16 <sup>a</sup> ±0.15   | 3.17 <sup>a</sup> ±0.12   |
| AST, U/L            | 36.95 <sup>a</sup> ±0.55  | 23.36 <sup>b</sup> ±0.47  | 23.33 <sup>b</sup> ±0.44  |
| ALT, U/L            | 42.50 <sup>a</sup> ±1.04  | 26.23 <sup>b</sup> ±0.18  | 26.17 <sup>b</sup> ±0.09  |
| Cholesterol, mg/dL  | 80.41 <sup>a</sup> ±0.36  | 65.34 <sup>b</sup> ±0.44  | 64.60 <sup>b</sup> ±0.62  |
| Triglyceride, mg/dL | 158.13 <sup>a</sup> ±0.79 | 101.33 <sup>b</sup> ±1.45 | 100.34 <sup>b</sup> ±1.46 |
| HDL, mg/dL          | 35.92 <sup>b</sup> ±0.11  | 41.88 <sup>a</sup> ±1.06  | 42.32 <sup>a</sup> ±0.69  |
| LDL, mg/dL          | 16.08 <sup>a</sup> ±0.51  | 5.37 <sup>b</sup> ±0.42   | 5.26 <sup>b</sup> ±0.25   |

Means in the same row within each classification bearing different letters are significantly different ( $P<0.05$ )

**Blood antioxidant and immune response in bee venom treatments as BV0, BV1 and BV2 kids:**

The effect of treating kids with BV0, BV1 and BV2 on the antioxidant status and immune response is summarized in Table (5). The data shows an increase ( $P>0.05$ ) in TAC levels in both BV1 or BV2 kids compared with those kids in BV0 group. However, antioxidant enzyme activities measured as GPx, SOD and CAT in kids treated with BV1 and

BV2 had the best ( $P<0.05$ ) values compared with BV0 kids. Both BV1 and BV2 kids had the lowest ( $P<0.05$ ) MDA compared to the BV0 group. Immune system was boosted in kids with BV1 and BV2, compared to control, which were shown by the higher ( $P<0.05$ ) of IgM and IgG levels compared to the BV0 kids.

**Table 5. Blood antioxidant status and immune response in bee venom treatments as BV0, BV1 and BV2 kids**

| Items        | Bee venom groups                |                           |                           |
|--------------|---------------------------------|---------------------------|---------------------------|
|              | BV0                             | BV1                       | BV2                       |
|              | <b>Blood antioxidant status</b> |                           |                           |
| TAC, µmol/ml | 1.93±0.03                       | 2.36±0.19                 | 2.43±0.23                 |
| MDA, nmol/ml | 0.51 <sup>b</sup> ±0.05         | 0.36 <sup>a</sup> ±0.02   | 0.35 <sup>a</sup> ±0.01   |
| SOD, IU/ml   | 6.65 <sup>b</sup> ±0.35         | 7.83 <sup>a</sup> ±0.15   | 7.90 <sup>a</sup> ±0.29   |
| GPx, IU/ml   | 6.12 <sup>b</sup> ±0.27         | 8.24 <sup>a</sup> ±0.15   | 8.36 <sup>a</sup> ±0.10   |
| CAT, IU/ml   | 72.46 <sup>b</sup> ±0.43        | 87.33 <sup>a</sup> ±0.28  | 87.77 <sup>a</sup> ±0.32  |
|              | <b>Blood immune response</b>    |                           |                           |
| IgG, mg/dL   | 335.30 <sup>b</sup> ±0.44       | 547.90 <sup>a</sup> ±2.15 | 551.20 <sup>a</sup> ±3.52 |
| IgM, mg/dL   | 36.11 <sup>b</sup> ±0.32        | 40.09 <sup>a</sup> ±0.29  | 40.99 <sup>a</sup> ±0.31  |

Means in the same row within each classification bearing different letters are significantly different ( $P<0.05$ ).

**Correlation coefficients between bee venom (BV) injection and FBW, DWG, FCR, PI, SC, TV and TC in kids:**

The correlation between BV0, BV1 and BV2 injection and productive performance as FBW, DWG, FCR, PI, SC, TV and TC in kids up to puberty are presented in Table (6). Positive correlations were observed between BV injections and all productive performance. Furthermore, FBW was positively ( $r=1.000$ ) and significantly ( $P<0.01$ ) correlated with

DWG. However, correlations were ( $P<0.05$ ) between BV injection FBW FCR ( $r=1.000$ ), TV ( $r=0.997$ ) and TC ( $r=0.999$ ). Furthermore, the FCR noticed highly significant correlation ( $P<0.01$ ) between FBW ( $r=1.000$ ) and DWG ( $r=1.000$ ). In addition, the relationship among TC and all testicular measurements such as SC and TV indicated more significant differences ( $P<0.05$ ) with SC ( $r=0.999$ ) and TV ( $r=0.999$ ). Also, the TV had significant correlation ( $P<0.01$ ) with SC measurements ( $r=1.000$ ).

**Table 6. Correlation coefficients between different studied traits (FBW, DWG, FCR, PI, SC, TV and TC)**

| Items | FBW | DWG     | FCR    | PI     | SC     | TV      | TC      |
|-------|-----|---------|--------|--------|--------|---------|---------|
| FBW   | 1   | 1.000** | 1.000* | 0.995  | 0.996  | 0.997*  | 0.999*  |
| DWG   |     | 1       | 1.000* | 0.995  | 0.996  | 0.997*  | 0.999*  |
| FCR   |     |         | 1      | -0.997 | -0.994 | -0.996  | -0.998* |
| PI    |     |         |        | 1      | 0.962  | 0.985   | 0.990   |
| SC    |     |         |        |        | 1      | 1.000** | 0.999*  |
| TV    |     |         |        |        |        | 1       | 0.999*  |
| TC    |     |         |        |        |        |         | 1       |

FBW = final body weight, DWG= daily weight gain, FCR= feed conversion ratio, performance index= PI, SC= scrotal conformance, TV =testes volume and testosterone concentration=TC,

\*Correlation is significant at the (P<0.05). \*\*Correlation is significant at the (P<0.01).

## DISCUSSION

Many studies described biological activities of bee venom (BV) components application in livestock nutrition (Frangieh *et al.*, 2019). Also, the BV has powerful antioxidant and apparently normalizes structure of the mitochondria through prevention of micro-vasculopathy (Baher and Abo Zeid, 2017).

### **Measurement of FBW, TFI, DWG, FCR, PI, MW and PEF in bee venom treatments as BV0, BV1 and BV2 kids:**

BV may have significant use in animal welfare. Hence, increasing of FBW and DWG as well as TFI was numerically higher in BV1 and BV2 kids, but not significance, which was reflected positively in FCR, PI, MW and PEF compared with BV0 kids. Similarly, Adel *et al.* (2022) who revealed that injecting rabbits with 0.3 mg /rabbit of BV twice a week from 5 to 10 weeks of age resulted in (P<0.05) higher FCR and FBW compared with the control group. Also, our results are in agreement with the finding of Han *et al.* (2009) who found that stinging or subcutaneously injecting with BV resulted in a net increase in FBW and DWG and survivability in piglets. Also, Han *et al.* (2013) found that supplementing pig and rat diets with BV melittin has high-quality products and therefore there were significant differences in FBW. Likewise, Rabie *et al.* (2018b) noticed that BV could be ascribed to its health benefits by prevention diseases in chickens. In the current study, both BV1 and BV2 kids had recorded the highest (P<0.05) values in FCR versus BV0 kids. Ali and Mohanny (2014) reported that the birds injected with 0, 0.5, 1.0 and 1.5 of BV observed FCR an increase up to 1.90, 1.83, 1.72 and 1.75, respectively. Melittin is the principal component in BV, it is the best stimulus to growth performance in newly born chicks under highly concentrated production environment in the broiler industry (Kimet *et al.*, 2019). Carpena *et al.* (2020) noticed that BV can increase productive performance by improving capillary permeability, blood circulation and lower blood pressure and reducing infectious diseases. BV has a significant antioxidant property against ionizing radiation which can adversely influences growth performance (Malek *et al.*, 2023).

Similarly, Elkomy *et al.* (2023) stated that rabbits received 2, 4 and 8 mg BV/ kg recorded BWG at 1520, 1578 and 1556g, FI at 5522, 5338 and 5290g and FCR at 3.52, 3.40 and 3.45 compared with BWG up to 1396g , FI at 5552g , FCR at 3.97 in control group, respectively. El-Banna *et al.* (2023) reported that supplementation of BV in drinking water had a substantial effect on productive performance included body weight, feed intake, antioxidative capacity. Finding of, Elkomy *et al.* (2023) who noticed that providing BV at 2, 4, and 8 mg/kg body weight /day in drinking water resulted in a significant increase in BW and a decrease in FI, resulting in improved FCR and carcass quality of rabbits.

### **Measurement of scrotal circumference, testes volume and testosterone level in bee venom treatments as BV0, BV1 and BV2 kids through sexual puberty phases:**

Puberty in kid goats has been described as the phase of body development during which the gonadotropic hormones are secreted in sufficient amounts to cause accelerating growth of genital organs and the appearance of secondary sexual characters hence, changes are accompanied by the ejaculation of the first sperm (Khalifa *et al.*, 2021). In the present study, kids in both BV1 and BV2 groups resulted in earlier sexual puberty compared to BV0 kids might be related to the highest metabolic components in BV which consumption with basal rations. These observations agree with the study of Nasir *et al.* (2013) who reported that reproductive functions such as reaching puberty and sexual maturity require the best quantity of feed consumption from puberty to maturity phases. Furthermore, Bompadre *et al.* (2014) reported that reduced quality of feed consumption during sexual development is attributed to less internal organ mass that negatively affect the normal sexual behaviors. The current results elucidated that the effects of BV on the organs by its contents of molecules that are able to modify the hormonal and neuronal activity. As well as, Florea *et al.* (2009) demonstrated that BV can intensified metabolic activity and increased neurosecretory activity in hypothalamic nuclei which influences with the hormonal secretory activity. Also, Zarrinnahad *et al.* (2018) found that the injection of



melittin stimulates pituitary gland to release gonadotropin hormones causing liberation and release FSH and LH to circulation which can have a positive effect on testicular development. Likewise, Melmed and Jameson (2015) explained that BV transported from the site of injection by the blood arrived at the pituitary portal system and superior hypo-physeal artery that facilitates the release of hypothalamic (its main function is to keep the body in a stable state called homeostasis) hormones. A more important sexual organs growth was also observed during administration of BV in experimental rats especially Sertoli cells in testicles (Tilincea and Florea, 2018). Confirmation by Florea *et al.* (2019) who revealed that the most important components molecules (mainly melittin and phospholipase A2) of the BV brought by the blood flow which could be taken into consideration that in turn contributed to testicular stimulation to produce more sexual hormones. According to El-Hanoun *et al.* (2021) also concluded that BV had significant positive impacts on some semen quality traits and sexual behavior in V-line bucks. Likewise, Elkomy *et al.* (2021) suggested that BV has an effective and safe alternative to sexual-stimulants needed to improve certain reproductive traits, immune response and health in rabbits. Also, the BV has been shown to boost buck semen quality including:- sperm concentration (35%), live sperm (6%) and fertility percentage (14%) when BV injected at 0.1–0.3 mg/kg according to Abd El-Aziz *et al.* (2023).

#### **Biochemical blood components in bee venom treatments as BV0, BV1 and BV2 kids:**

In the current study, increasing blood total protein concentration in BV1 or BV2 kids compared with BV0 kid was also reported by Elkomy *et al.* (2021) who suggested that injected BV could increase blood total protein. Higher levels of blood globulin in BV1 or BV2 kids than BV0 kids may be related to active peptide content in bee venom. The present results were in agreement with those of El-Hanoun *et al.* (2020) and Elkomy *et al.* (2021) who demonstrated that the BV could be increased blood globulin concentration more ( $P < 0.05$ ) than the control group. On the other hand, Han *et al.* (2013) stated that more ( $P < 0.05$ ) impacts on total protein, albumin and globulin in rat and pig who received bee venom than control group.

The reduction in AST and ALT enzymes activities for BV1 or BV2 kids were mentioned by El-Hanoun *et al.* (2020). Likewise, Elkomy *et al.* (2021) defined that BV injected subcutaneously with male and female adult rabbits had caused a significant reduction in liver enzyme activities for AST (16%) and ALT (36%). The reduced cholesterol, LDL and triglyceride in BV1 and BV2 kids were obtained in current study were similar to the findings in mice (Mousavi *et al.*, 2012), in pig (Han *et al.*, 2013), in broiler (Ali and Mohanny,

2014) and in rabbits (El-Hanoun *et al.*, 2020 and Elkomy *et al.*, 2021).

Similarly, Elkomy *et al.* (2023) recoded that when rabbits were received BV at 0, 2, 4 and 8 mg/kg body weight had cholesterol concentration at 80.33, 65.33, 60.67 and 59.33 mg/dL, triglyceride level up to 157.50, 100.00, 92.00 and 90.33 mg/dL and LDL reached to 14.18, 5.36, 4.60 and 4.27 mg/dL, respectively. Also, the previous authors indicated that an improvement in beneficial type of cholesterol as HDL concentration which is agreement with our study, it was 39.97, 38.33 and 36.33 mg/dL in 2, 4 and 8 mg of BV, respectively compared with 34.68 mg /dL in control.

#### **Blood antioxidant status and immune response in bee venom treatments as BV0, BV1 and BV2 kids:**

Our results revealed that the effect of injected BV on growing kids could be explained by the levels of TAC, GPx, SOD, IgG and IgM, but the decreasing level of MDA level compared with control kids (Ali *et al.*, 2014, El-Hanoun *et al.*, 2020 and Elkomy *et al.*, 2021). Confirmation by Kim *et al.* (2019) who indicated that BV has the most pharmacological antioxidant properties, can protect against cellular injury and consequently reducing oxidative damage to either lipids or proteins. Likewise, Kocyigit *et al.* (2019) who revealed that increasing total antioxidant status, total oxidant status and oxidative stress index in rats received BV compared with control rats. Also, El-Banna *et al.* (2023) noticed that BV can increased concentration of antioxidant levels by plays an endogenous antioxidant protection system by removing free radicals and maintaining the intracellular redox balance. In the same vision, Elkomy *et al.* (2023) who recorded that BV levels at 0, 2, 4 and 8 mg/kg body weight has TAC up to 1.91, 2.42, 2.68 and 3.01  $\mu\text{mol/ml}$ , MDA was 0.42, 0.33, 0.28 and 0.29 nmol/ml, SOD reached to 6.38, 7.32, 8.41 and 7.80 IU/ml, Gpx was 6.09, 7.84, 8.12 and 8.56 IU/ml, CAT was 71.16, 87.75, 83.43 and 89.77 IU/ml, IgG was 331, 645, 525 and 415 mg/dl and IgM up to 35, 53, 43 and 40 mg/dl, respectively. Typically, BV is containing number of organic and inorganic substances which together determine an antioxidant effect on cells protection from damaging (Matkivska *et al.*, 2024).

#### **Correlation coefficients between different studied traits (FBW, DWG, FCR, PI, SC, TV and TC)**

As would be expected from the current study, all measurements between TV and FBW and SC and TC were positively correlated with one another. Mahdy *et al.* (2023) who revealed that significant correlation coefficients between TV and LBW, SC and TC up to  $r=0.979$ ,  $r=0.999$  and  $r=1.000$ , respectively. The LBW could provide a useful estimate of DWG ( $r=1.000$ ), TV ( $r=0.997$ ) since, its correlations with the TC ( $r=0.999$ ) were the highest. Similar results were explained to (Behery *et al.*, 2015, Elkomy *et al.*, 2021, Adel *et al.*, 2022 and El-Banna *et al.*, 2023).

## CONCLUSION

Generally, injection of bee venom (BV) up to 250 or 500mg / kid / weekly may positively affect productive performance, blood characteristics and health status as immunological and antioxidative responses of weaning kids to sexual puberty.

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## تأثير سم نحل العسل على أداء نمو الجداء الزرايبي من الفطام إلى البلوغ الجنسي

عز الدين ابراهيم خليفة<sup>١</sup>، سارة ابراهيم جراوش<sup>٢</sup>، عبد الحميد أحمد عواد ابراهيم جمعة<sup>١</sup>، أيمن محمد عبد الفتاح غنية<sup>٢</sup>، عمر أحمد متولي<sup>٢</sup>، طارق مسلم محمود مهدي<sup>١</sup>

١ - معهد بحوث الانتاج الحيواني، قسم بحوث تربية الأغنام والماعز، ٢ - معهد وقاية النبات، قسم بحوث نحل العسل

أجريت هذه الدراسة لدراسة تأثير حقن سم النحل على أداء النمو للجداء الزرايبي حتى البلوغ الجنسي. تم تقسيم اثني عشر جدي بعد الفطام عمر ٩٠ يوماً بمتوسط وزن حي  $10.50 \pm 0.54$  كجم عشوائياً إلى ثلاث مجموعات تجريبية (العدد=٤ جداء/معاملة). تم تغذية جداء المجموعة الأولى بنظام غذائي أساسي بالإضافة إلى حقنهم أسبوعياً ١,٠ مل من الماء المقطر بدون سم نحل (BV0) الكونترول. تم تغذية جداء المجموعة الثانية والثالثة بنفس النظام الغذائي الأساسي وتم الحقن ١,٠ مل من الماء المقطر /أسبوعياً /جدي كل مل يحتوي إما على ٢٥٠ أو ٥٠٠ ملجم من سم نحل BV لتكوين BV1 أو BV2 على التوالي. تم تقدير معدل النمو وقياس محيط كيس الصفن وحجم الخصيتين ومستوى هرمون التستوستيرون وقياسات الدم خلال مراحل البلوغ الجنسي. واهم النتائج التي تم الحصول عليها أن حقن سم النحل للجداء (BV1 أو BV2) كان له تأثير على أداء النمو أعلى بكثير من جداء BV0 وقد أسفرت النتائج أن جداء BV1 وBV2 أعطت أفضل قياس لمحيط كيس الصفن وحجم الخصية ومستوى هرمون التستوستيرون خلال مراحل البلوغ الجنسي مقارنة مع الجداء BV0 أيضاً أظهرت خصائص الدم تحسن عند مستوي معنوية ( $P<0.05$ ) لدى جداء BV1 أو BV2 مقارنة مع جداء BV0 علاوة على ذلك كان هناك تأثير إيجابي على الجداء في BV1 و BV2 على مؤشرات مضادات الأكسدة وانخفاض المالونديالدهيد (MDA) بشكل ملحوظ عند مستوي معنوية ( $P<0.05$ ) مقارنة مع جداء BV0.

**الخلاصة:** سم النحل هو مادة طبيعية تعمل على تعزيز أداء النمو وتقليل أوقات مراحل البلوغ الجنسي وتحسين الاستجابات المناعية ومضادات الأكسدة لدى الجداء في مرحلة النمو المختلفة.