SHORT COMMUNICATION

Ovulation inducement potential of a polyherbal supplement in rabbit does raised under the tropical Humid conditions

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Abstract

Ovulation is a critical reproductive event preceeding conception and it determines litter size and overall fertility in the female animals. In rabbits, an induced-ovulating species, in which mating trigers ovulation in the female, an alternative means to induce ovulation is absolutely necessary under artificial insemination breeding systems. Therefore, welfare-compatible ovulation-inducement methods are important for improving reproductive outcomes and ensuring sustainable rabbit breeding performance. Janova®, a polyherbal product previously indicated to enhance reproductive performance in ruminants by inducing ovulatory oestrus, was evaluated for its ability to induce ovulation in rabbit does. Forty mixed-breed mature does (2.5–3.0 kg) were allocated to four treatments in a completely randomized design: T1, natural mating with no dietary supplement; T2, diet supplemented with 2.0 g Janova®/kg feed followed by insemination with extended semen; T3, diet supplemented with 4.0 g Janova®/kg feed and inseminated similarly; and T4, no dietary supplement but inseminated with semen extended using a GnRH-containing diluent (MRAbit®). Supplements were administered for 7 days before insemination. Blood samples collected at mating/insemination and at 60 and 120 minutes were analysed for FSH and LH. Statistical results showed no significant differences in FSH or LH concentrations over time within treatments, and no clear LH surge indicative of ovulation was detected. Conception and kindling occurred only in unsupplemented groups (T1 and T4), with no differences in gestation length, litter size or litter weight between them. The study demonstrates that oral administration of Janova® at 2.0 g or 4.0 g per kg feed for 7 days did not induce ovulation in rabbit does and thus could not be used as a possible ovulation inducer for rabbits.

Key words: Rabbit does, Janova®, Ovulation induction, Reproductive hormones.

Running title: Polyherbal Supplement for Ovulation Inducement in Rabbit Does

Potentiel d'induction de l'ovulation d'un complément polyherbacé chez les lapines élevées dans des conditions tropicales humides

Résumé

L'ovulation est un événement reproductif essentiel qui précède la conception et détermine la taille de la portée et la fertilité globale chez les animaux femelles. Chez les lapins, une espèce à ovulation induite, chez laquelle l'accouplement déclenche l'ovulation chez la femelle, un moyen alternatif pour induire l'ovulation est absolument nécessaire dans les systèmes d'élevage par insémination artificielle. Par conséquent, des méthodes d'induction de l'ovulation compatibles avec le bien-être animal sont importantes pour améliorer les résultats reproductifs et garantir des performances d'élevage durables chez les lapins. Janova®, un produit polyherbacé précédemment indiqué pour améliorer les performances reproductives des ruminants en induisant l'œstrus ovulatoire, a été évalué pour sa capacité à induire l'ovulation chez les lapines. Quarante lapines adultes de race mixte (2,5-3,0 kg) ont été réparties en quatre groupes de traitement selon un plan complètement randomisé : T1, accouplement naturel sans supplément alimentaire ; T2,

alimentation complétée par 2,0 g de Janova®/kg d'aliment, suivie d'une insémination avec du sperme dilué ; T3, alimentation complétée par 4,0 g de Janova®/kg d'aliment et insémination similaire ; et T4, sans complément alimentaire, mais insémination avec du sperme dilué à l'aide d'un diluant contenant de la GnRH (MRAbit®). Les compléments ont été administrés pendant 7 jours avant l'insémination. Les échantillons sanguins prélevés au moment de l'accouplement/de l'insémination et après 60 et 120 minutes ont été analysés pour déterminer les taux de FSH et de LH. Les résultats statistiques n'ont montré aucune différence significative dans les concentrations de FSH ou de LH au fil du temps au sein des traitements, et aucun pic de LH indiquant une ovulation n'a été détecté. La conception et la mise bas n'ont eu lieu que dans les groupes non supplémentés (T1 et T4), sans différence entre eux en termes de durée de gestation, de taille de la portée ou de poids de la portée. L'étude démontre que l'administration orale de Janova® à raison de 2,0 g ou 4,0 g par kg d'aliment pendant 7 jours n'a pas induit d'ovulation chez les lapines et ne peut donc pas être utilisée comme inducteur d'ovulation chez les lapines.

Mots clés : Lapines, Janova®, Induction de l'ovulation, Hormones reproductives.

Introduction

Ovulation is a critical reproductive event that determines the timing and success of conception, embryonic development, as well as overall fertility in mammals. In rabbits (an induced-ovulating species), the absence of a natural pre-ovulatory surge can limit reproductive efficiency, especially under controlled breeding systems. Understanding and improving ovulation inducement therefore remains essential for optimizing fertility and enhancing productivity. Consequently, research on reliable, welfare-compatible ovulation-inducement methods is particularly important for improving reproductive outcomes and ensuring consistent, sustainable breeding performance of rabbits.

Janova® is a polyherbal product indicated for improved reproductive performance, particularly in ruminants. Its ability to induce ovulatory oestrus was reported for the cow (Srivastava, 1997; Bawaskar *et al.,* 2017; Sutaria *et al.,* 2019), goat (Patil *et al.,* 2010) and buffalo (Jyothi *et al.,* 2020). This ability was attributed to the constituent herbs which possess gonadotropin-like activities and thus able to synchronize the release of reproductive hormones (FSH, LH and oestradiol) for the ovulatory oestrus to be induced (Srivastava, 1997).

Janova® contains; *Citrullus collocynthus* (bitter cucumber), *Piper longum* (long pepper plant), *Piper nigrum* (black pepper) and *Zingiber officinale* (ginger). Gingerol – 6 and piperine are two important bioactive compunds from this mixture with proven potency for ecbolic, antimicrobial, anti-inflammatory, analgesic and muscular tissue stimulation (Wang *et al*., 2014).

As a neuro-induced ovulator, ovulation induction through pituitary stimulation is a compulsory step in artificial insemination protocol for rabbit does. This is usually achieved through intramuscular injection of GnRH (or its analogues) (Rebollar *et al*., 2012; Mobarak *et al.,* 2015), or same added to the semen insemination dose (Quintela *et al.,* 2004) at the time of insemination to induce ovulation in the does to be inseminated. Janova® is indicated for oral administration and there is no reported study suggesting that oral dosage of ovulation inducing agent can be effective in induced ovulator like rabbit. Janova®, especially as a herbal preparation, is therefore a candidate for non injectable source of ovulation induction which is yet to be tried in the rabbit does. Thus, the aim of this study was to determine the potential of Janova® as oral feed supplement to induce ovulation in rabbit does.

Materials and Methods

Animals and experimental design

A total of 40 rabbit does (crosses of New Zealand White, Chinchila, California and Dutch belted breeds) were randomised by body weight equally

into 4 treatments. The does varied in parity (0 – 3), age (8 – 16 months) and body weight (2.5 – 3.0 kg). The treatments includes; T1 (control): does naturally mated to bucks with no herbal supplement in the diet; T2: does inseminated after dietary supplementation with 2.0 g of Janova® per kg feed; T3: does inseminated after dietary supplementation with 4.0 g of Janova® per kg feed; while T4: does inseminated with semen with diluent that contained GnRH analogue (MRAbit®), animals received no supplement in diet. The Janova dosage used was extrapolated from the recommended dosage for small ruminants (100g/30-50kg body weight). For T2 and T3 feed supplementation was done for 7 days. The study received ethical clearance of the Department of Animal Science and of Obafemi Awolowo University, Ile-Ife, Nigeria.

Breeding of Does

Natural mating and artificial insemination of treatment does were performed before 10.00 am same day. Semen was collected from fertile bucks using Ola Improvised Rabbit Artificial Vagina (OLIRAV) protocol (Ola, 2016). The pooled semen for AI was divided among treatment groups 2, 3 and 4. For treatments 2 and 3, the semen was extended with Kubus mra bit standard rabbit extender while the semen for treament 4 was extended with Kusu mra bit plus (containing GnRH analogue). Each inseminated doe received an insemination dose not less than 20×10^6 spermatozoa in a volume of 0.5 mL.

Data Collection

Data was collected on plasma concentrations of luteinizing hormone (LH) and follicle-stimulating hormones (FSH), conception rate, gestation length, kindling rate, litter size, average kit weight and litter weight at kindling. For LH and FSH assay, 2 mLs of blood sample were collected at 0, 60, and 120 minutes time periods post AI, into anticoagulant treated sample tubes by puncturing the central marginal ear vein from three animals per treatment. The blood samples were then centrifuged at 3000 rpm for 15 minutes to retrieve the plasma which were stored at -20 ^{0}C until analyses. The hormonal assay was carried out at an Haematology Laboratory using the ELISA procedure.

Mated does were palpated for conception on day 15 – 18 post mating and pregnancy was monitored until the day of kindling when the litter information was obtained.

Statistical Analysis

The data collected were analysed with SAS® software using One-way analysis of variance or T-test where only two treatments were involved and significant differences among means were separated with Duncan's Multiple Range Test and differences considered significant at $p<0.05$.

Results and Discussion

Plasma concentrations of FSH and LH

The plasma level of FSH is indicative of follicular growth within the ovary while a surge in LH especially post mating in rabbit does is usually indicative of ovulation. The plasma level of FSH as shown in Figure 1 ranged between 35 – 48 ng/dL and there were no significant differences among the values obtained at the three different time periods in treatments 1, 2 and 3. But in treatment 4 FSH level at 120 minutes post mating was significantly lower than the level 60 minutes earlier. Similarly, for LH, plasma levels (Figure 2) were not different among time periods within the four treatments except in T4 where values at 60 minutes post mating was higher than values obtained 60 minutes later ($p<0.05$). LH values ranged between 15 – 30 ng/dL.

A consistent FSH levels indicated normal pre-ovulatory ovarian activity in all the treatment groups. A surge in plasma LH level is expected within 60 – 120 minutes following exogenous administration of GnRH or its analogue to rabbit does, either by parenteral (Rebollar *et al*. 2012) or intravaginal (Quintela *et al*., 2004) route, to indicate ovulation has been induced. A clear LH peak was not established for the current study indicating the inability of Janova oral supplement

to cause ovulation induction in the rabbit does. The conception results as shown in Table 1. further confirmed this assertion.

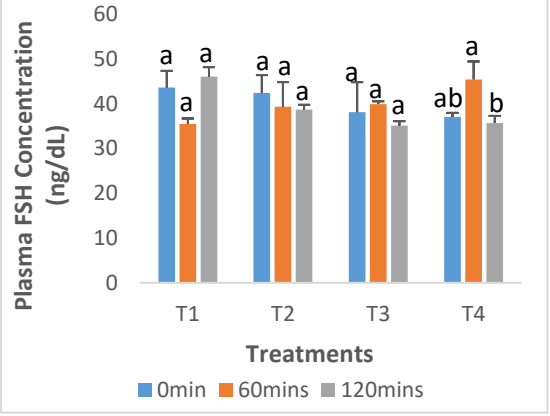

Figure 1: Plasma concentration of FSH at different time period in mated does

[ab]Columns within same treatment with different letters differ significantly ($p<0.05$, Duncan test).

[ab]Columns within same treatment with different letters

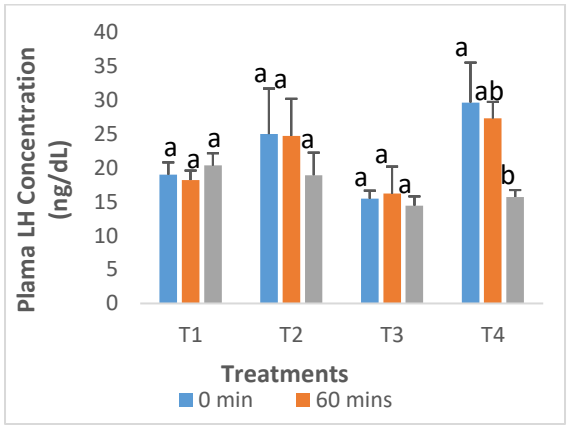

Figure 2: Plasma concentration of LH at different time periods in the mated does

[ab]Columns within same treatment with different letters differ significantly ($p<0.05$, Duncan test).

Gestation and litter parameters

Table 1 presents the pregnancy related parameters as obtained in the different experimental groups. After randomization, does average body weights were not different among the treatment groups. Only the animals in the group bred by natural mating (T1) and those that received semen that contained GnRH analogue (T4) conceived. In other words, animals in the group that received Janova supplemented diets (T2 and T3) did not conceive. Thus, only T1 and T4 were statistically compared by T test and result showed that they had similar conception rate, kindling rate, gestation length, litter size, litter weight and average kit weight at birth.

Janova had been reported to successfully cause anoestrus cows (Bawaskar *et al*., 2017) and buffalo (Jyothi *et al*., 2020) to exhibit oestrus and become bred. Janova may have stimulated follicular growth in the rabbit but not strong enough to stimulate gonadotrophin rise needed for ovulation induction. This may not be unconnected to the different digestive system of the rabbit compared to the ruminants, as well as the rabbits being neuro-induced ovulator. Therefore, a detailed nutritional physiological mechanism may be required, which will assist to establish appropriate dosage and actual period that LH peak is possible, if indeed Janova has gonadotrophin stimulatory effect in different categories of animals, including rabbits.

Table 1: Gestation and litter parameters in mated experimental does

Parameter	Treatments					
	T1	T2	T3	T4	SE	Prob.
Rabbit does, No.	10	10	10	10		
Doe Body weight (g)	2,297.00	2,347.90	2,356.10	2,382.10	78.21	0.90

Conception rate (%)	80.0	0	0	70.0		
Kindling rate (%)	60.0	0	0	50.0		
Gestation length (d)	31.8	0	0	32.7	0.50	0.26
Litter size (No)	5.40	0	0	6.33	0.31	0.16
Litter weight (g)	199.40	0	0	196.00	0.57	0.95
Average Kit weight (g)	37.2	0	0	30.7	2.62	0.26

0 = no conception or litter obtained.

Conclusion

Oral administration of Janova® as supplement in rabbit doe diet at the rate of 2.0 g and 4.0 g per kg feed for 7 days failed to induce ovulation in the rabbit does and thus could not be used as a possible ovulation inducer for rabbits..

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